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POTASH SHALE AS A SOURCE OF POTASSIUM FOR GROWING PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 405

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(WITH NINE FIGURES)

Introduction

Numerous attempts have been made to manufacture profitably soluble potassium fertilizer from potash rocks. While these rocks were our least source of supply during the emergency caused by the late war, they contain our greatest quantity of potassium because of their wide distribution. Ross (25) says that while the United States has the greatest phosphate mines in the world, it has twenty times more potash. Most of it, however, is confined in these difficultly soluble rocks of low grade, none of them over 10 per cent potash. The five principal forms of rock potash are orthoclase feldspar, greensand, alunite, leucite, and shale. They all belong to the group of silicate rocks except alunite, which is a hydrous potassium aluminum sulphate. GARDINER (11) states that 128 patents on processes for treating silicate rock for the extraction of potash were issued between 1904 and 1917. The European deposits being richer and very soluble, American manufacturers cannot compete with the foreign product, except when the rocks can be used in other forms of manufacture and the potash obtained as a by-product.

Many experiments have been made using potash rocks pulverized and applied directly to the soil as a source of potassium for plants.

There are some advantages in using mineral fertilizers in this form. While they require heavier initial application, they are cheaper and do not leach out so readily. In the case of potassium these less soluble rock fertilizers are never harmful, while heavy applications of the soluble forms sometimes prove injurious because of their bad effects on soil structure (13), because of impurities they contain as by-products of other forms of manufacturing (22), and because they may prevent the formation of nodules on the roots of legumes (20).

Most of the earlier experiments with orthoclase feldspar seemed to give favorable results, as for example, those of MAGNUS, AITKEN, NILSON, and BALLENTINE, all quoted by DE TURK (9), who likewise obtained promising results. However, most of the later investigators reported unfavorably. Among these are HARTWELL and PEMBER (15), BROOKS and GASKILL (7), VANATTA (34), and PRIANISCHNIKOV (24).

Certain greensands or glauconites of the Atlantic Coast have long been used locally without pulverizing. TRUE and GEISE (31), SKEEN (28), and BLAIR (4) found them useful as sources of potassium. Much of the greensand is so low (1.6–5.8 per cent) in potassium content, however, that it requires very heavy application.

Comparatively few experiments have been carried out with potash shales, but most of them have shown considerable potassium available for growing plants. STEWART (29) tried a Nevada shale and compared it with leucite, alunite, and kainit, finding that in potassium-deficient peat this shale produced better growth than any other potash carrier tested. PARR and others at the Illinois Experiment Station (23) obtained a better growth of corn with 2.68 tons of Union County shale than with five-sixths of a ton of kainit per acre. On the other hand, Dixon County shales were of little benefit. This difference in fertilizer value of the two shales is attributed to a difference in the form in which the potash is held in each. Sixty-two per cent of the potassium in the Union County shale was made soluble by treatment with concentrated sulphuric acid, while the acid had little effect upon the solubility of the potassium in the Dixon County shale. They think that the potassium made soluble in the Union County shale was in the glauconitic form. SCHMITT (26) secured a good response to the pulverized Decorah Shale of Minne-

sota. Sixty per cent of the potassium in this shale was made soluble by the concentrated sulphuric acid treatment.

The largest outcrop of potash shales known in this country is near Cartersville, Georgia. The State Geological Survey (**19**) reports it as approximately half a mile wide and 15 miles long. It has been drilled 400 feet without reaching bottom. STOCKETT (**30**) names the three great potential sources of potash in the United States as the leucite hills of Wyoming, the greensands of the Atlantic Coast, and the potash shales of Georgia. Possibly there may be added to these the deep potash deposits of western Texas which have been reported from the logs of oil wells. The Cartersville shale contains 6.6–8 per cent potassium, and has been used in this investigation.

Methods

In all, twelve kinds of plants were grown, in 676 containers. Some were grown in quartz sand and others in potash-deficient soils, of

TABLE I

POTASSIUM CONTENT OF SHALE AND OF SOILS, AND LIME REQUIREMENT OF SOILS

MATERIAL	POTASSIUM PERCENTAGE	LIME REQUIREMENT FOR ALFALFA (TONS PER ACRE)
Cartersville shale	7.9	
Peat from University of Wisconsin	0.25	2
Peat from Coddington Farm	0.51	2
Plainfield sand	0.70	2
Hazel Crest sand	1.74	4

which four types were used. The sample of shale, kindly furnished by Mr. J. T. NORRIS of Cartersville, was of light olive drab color, rather soft, and easily pulverized. Table I shows the potassium content of the shale and the soils, together with the lime requirements of the soils as indicated by the Truog test.

QUARTZ SAND

The quartz sand used was no. $3\frac{1}{2}$, manufactured by the Wausau Quartz Company and claimed by them to contain practically no plant nutrients. The shale was ground in a mill and in a mortar

until it passed a 100-mesh sieve. Six-inch earthenware pots were washed and dipped for 2 minutes into melted paraffin. Approximately 1415 cc. of quartz sand weighing 1892 gm., and the individual portions of shale were mixed well and poured together into each pot. The shale was added on the basis of tons per acre, assuming a 15 cm. depth of soil weighs 824,155 kg. per acre. The plants grown in quartz sand were white sweet clover, tomatoes, corn, squash, and cotton. In all except the squash, seven rows of pots were used and each row contained eight pots. Each of the rows from no. 2 to no. 6 were watered with Shive's solution $R_5C_2(27)$, modified by using an equivalent of PO_4 in $Ca(H_2PO_4)_2$ in place of KH_2PO_4. The solutions were made up in tap water, which the analysis of the Board of Health of the City of Chicago showed to contain only 0.9 parts per million of potassium. Row no. 1 was watered with tap water and row no. 7 with Shive's solution complete. The amount of potassium in the tap water was thought too low to affect the results. The solutions had to be used abundantly because of the heat in the greenhouse, so they were used one-fifth strength. The squash plants grew four weeks, the corn and tomatoes six weeks, and the cotton and sweet clover ten weeks.

Peaty soils

Peaty soils were used because they are often deficient in potassium, and because it was thought that the large amount of organic matter with its naturally occurring acids and its attendant increase in carbon dioxide and bacterial action in nitrification might increase the solubility of the potassium in the shale, as was indicated by the work of Ames and Boltz (1). The first peat used was a virgin soil from the University of Wisconsin marsh, furnished by the State Department of Soils through the courtesy of Dr. Geib. It will hereafter be designated as the University of Wisconsin peat. The second peat was obtained from the Coddington State Experiment Farm in Portage County, Wisconsin, kindly furnished by Dr. E. Truog. It will be called the Coddington peat. These peats are chocolate brown in color when dry but brownish black when wet. They contain many partially decayed roots and stems, and were put through a 6-mm. mesh sieve to remove the coarsest materials. After this each was thoroughly mixed. Corn and clover were grown in the

University of Wisconsin peat in 6-inch tinned-fruit cans coated inside with melted paraffin and having five 6-mm. holes in the bottom of each. Tomato plants followed the corn in the same cans of soil without addition of fertilizer. Buckwheat and oats were grown in the Coddington peat in paraffined 6-inch pots. The corn and clover were grown in quintuplicate in large south windows from January to May, with the rows of the series running at right angles to the plane of the windows. The tomatoes were grown in triplicate in the same situation. The buckwheat and oats were grown out of doors (in Chicago) on a bench during July and August.

Sandy soils

Sandy soils were used for comparison with the peats and quartz sand. Plainfield sand, a soil deficient in potassium, was obtained from the University of Wisconsin. The second sandy soil was collected from Hazel Crest, Illinois, about 20 miles south of Chicago. It was taken from a field lying next to the west side of the Illinois Central Railroad right-of-way, just south of 171st Street. It was once part of the lake bed of the old Calumet Stage of Lake Chicago, and lies about a quarter of a mile inside of the old Calumet Beach. The first test with buckwheat seemed to indicate that it was deficient in potassium, but later results showed that it was not potassium-deficient for corn. It is a dark brown sandy loam with a high acidity, and since it has not been named by a soil survey, it will be designated as Hazel Crest sand.

Alfalfa and winter wheat were grown in the Plainfield sand, the former in triplicate in paraffin-coated tin cans, and the winter wheat in single 3-gallon stone crocks with a 12-mm. hole in the bottom of each. The wheat was grown from December 1 to May 3 and the alfalfa from January to May, both in south windows.

Alfalfa and medium red clover were grown in triplicate in the Hazel Crest sand in the same sort of 6-inch cans and in south windows from January to May. Corn was also grown in the Hazel Crest sand, but out of doors during July and August in 1-gallon glazed crocks with a 12-mm. hole in each. The crocks were set on boards in the bottom of a hotbed without artificial heat and without glass except during rain. The fertilizers were added on a basis simi-

lar to that used in the quartz sand. As indicated in the tables, the University of Wisconsin peat was limed at a rate of 2.5 tons per acre with Missouri limestone fine enough to pass a 60-mesh sieve. This limestone was found to neutralize 97.3 per cent as much dilute nitric acid as did the chemically pure precipitated $CaCO_3$ used in the outdoor experiments. One-half of each row of oats and buckwheat grown in the Coddington peat was treated with the equivalent of 2 tons per acre of chemically pure precipitated $CaCO_3$. The Plainfield sand was limed for alfalfa at the rate of 2 tons per acre of Missouri limestone, as was also one-half of the crocks of the same soil in which was grown winter wheat. Most of the Hazel Crest soil was given 4 tons of limestone to the acre. All of the plants except those grown in the quartz sand were watered with distilled water.

Use of gypsum and limestone with shale

It has been claimed by some gypsum producers and some soil workers (**2, 8, 35**) that gypsum increases the availability of insoluble forms of soil potassium; therefore combinations of gypsum and potash shale were used. In the quartz sand and in the University of Wisconsin peat, some of the rows received equal quantities of pulverized shale and chemically pure $CaSO_4$ mixed with the soil at the same time. In all the other experiments, one row of pots was treated with shale that had been mixed thoroughly with about 20 per cent of its own weight of $CaSO_4$ and roasted at red heat for 30 minutes. In the Coddington peat and in the two sandy soils, one row of pots with each kind of plant received the shale roasted with 15 or 20 per cent of its weight of $CaCO_3$. In the sandy soils, two additional rows of pots were treated with the shale which had been autoclaved at 20 pounds steam pressure for 30 minutes with gypsum and $CaCO_3$ respectively. To learn whether any of the soils were deficient in sulphur, and therefore responded to the gypsum, two extra control rows were grown. One was usually numbered 1b, and it was untreated the same as the control except that it received gypsum. The other row received KCl and gypsum.

Chemical analyses

In order to determine whether the potash shale increased the intake of potassium in the plants grown on it, the dry tops of five sets

of plants were analyzed for potassium. A sodium cobalti-nitrite method was used, which was originally perfected by BOWSER (6) and modified by KOCH. It consisted in oxidizing the tissue by the wet method, using concentrated sulphuric acid with a few drops of fuming nitric acid. The acid was then evaporated to dryness over a low flame, and the potassium salt with traces of other sulphates heated to redness to drive off any trace of acids or of ammonium sulphate. The residue was taken up in about 2 cc. of ammonium-free water, acidified with 1 cc. of glacial acetic acid, and precipitated with a strong solution of sodium cobalti-nitrite. It was evaporated on a steam bath to a paste to insure complete precipitation. The paste was stirred with approximately 25 cc. cold water and the precipitate filtered out on an asbestos pad in a Gooch crucible. The pad and precipitate were transferred to a beaker, stirred with 25 cc. water, then acidified with 5 cc. of approximately 50 per cent sulphuric acid. This was boiled with an excess of N/20 potassium permanganate solution and decolorized with N/20 oxalic acid. Enough of the same potassium permanganate was used to bring the color back to a faint pink. The difference between the amount of permanganate used and the amount of oxalic acid gave the amount of permanganate required to oxidize the potassium-sodium cobalti-nitrite precipitated. One cc. of N/20 $KMnO_4$ is equivalent to 0.0003595 gm. potassium. When tested in comparison with the Lindo-Gladding method on pure solutions and on plant tissues, the cobalti-nitrite method was found to run approximately 1 per cent higher, but to be more constant in the presence of the impurities in the plants, as was found by DODD (10). HAMID obtained better results with the cobalti-nitrite method than with the perchloric acid method (14). The slightly higher results, being constant, do not affect the comparison of the potassium content in the plants.

Nine of the thirteen rows of corn grown in Hazel Crest sand were analyzed for nitrogen and carbohydrates, as well as for potassium. This corn tissue was preserved in approximately 80 per cent alcohol to which was added a little $CaCO_3$. To separate the soluble from the insoluble material, the alcohol was drained off and the tissue extracted with a Wylie or a Landsiedl extractor for 4 hours. The tissue was dried in a 100° oven and ground in a mortar to pass a 40-mesh sieve, and extracted five times with hot water. After this it was

extracted in approximately 75 per cent alcohol for 12 hours. Young plant tissues like corn contain so little ether-soluble materials that it was thought unnecessary to carry out the usual ether extraction. The preserving alcohol and the water and the alcoholic extracts were poured together and made up to volume. Total solids were determined in the usual way. The extract and tissue were next analyzed for total nitrogen by the Gunning method, modified to include nitrate nitrogen (3). Each was then analyzed for nitrate plus ammonia nitrogen by the Official Zinc-iron Method (3) and recorded as such in the table. The percentage of ammonia plus nitrate nitrogen was subtracted from the total nitrogen in the extract and the difference recorded as soluble organic nitrogen. These analyses were repeated with the tissue and the difference recorded as insoluble organic nitrogen.

Reducing sugars were estimated by the Official Munson and Walker general method (3) and with the volumetric permanganate method with the solution standardized against sodium oxalate. Non-reducing sugars were inverted for 7 minutes at 70°, making the total time of heating 10 minutes, with 5 cc. concentrated HCl (sp. gr. 1.19) in 75 cc. solution. The amount of non-reducing sugars was found by difference between the percentages of reducing sugars before and after hydrolysis. Polysaccharides were estimated in the extract and in the tissue as directed in the Official Methods for starch (3) and recorded as invert sugars. No clearing agent was used, for trials with and without a clearing agent gave such slight differences that they were thought to be within the experimental error. Similar possibilities are suggested by LOOMIS (18), and similar results were obtained with and without clearing agents by MORRIS and WELTON (21).

Results

QUARTZ SAND

Table II and figs. 1, 2, and 3 show the results obtained with quartz sand. The growth responses are indicated in the table in percentages of gain over the control. The variation in the responses of different kinds of plants to potash fertilizer is very striking. With the complete nutrient solution, there was an increased growth of

11.1 per cent in sweet clover and of 119.8 per cent in corn. The increases with potash shale varied from 14.8 to 62.1 per cent, omitting the squash which was tested with only 1.5 tons per acre. The ratios between the gains in potash shale and the gains in complete Shive's solution are just as variable, but this ratio is largest when the gains are smallest. With the corn, the shale caused about 40 per cent as great a response as was produced by the complete Shive's solution; with tomatoes, 65 per cent; cotton, 120 per cent; and sweet

TABLE II

PLANTS GROWN IN QUARTZ SAND; INCREASED GROWTH GIVEN IN PERCENTAGE GAIN OVER CONTROL

No.	Shive's solution used	Tons of shale	Corn*		Tomatoes*		Squash*		Cotton*	Sweet clover*
			Green	Dry	Green	Dry	Green	Dry	Green	Green
2.....	No K	0.0	control							
3.....	No K	0.5	7.5	18.3	33.6	Lost	.....	.....	9.8	14.8
4.....	No K	1.5	28.6	43.2	22.1	Lost	12.5	11.8	5.7	14.2
5.....	No K	3.0	29.8	43.2	14.0	Lost	.....	.....	19.5	12.0
6.....	No K	6.0	47.4	62.1	61.7	Lost	.....	.....	17.1	1.9
7.....	With K	0.0	119.8	97.4	90.8	Lost	61.8	64.5	15.5	11.1

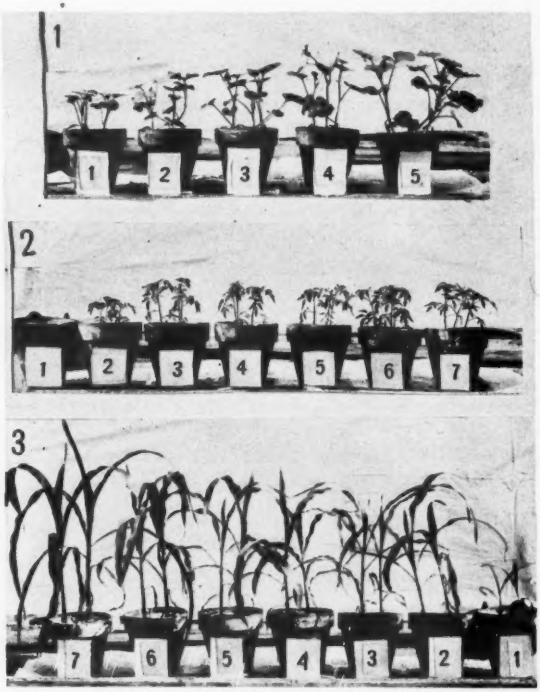
* The squash grew 4 weeks, the tomatoes and corn 6 weeks, and the cotton and sweet clover 10 weeks.

clover, 150 per cent. Some of this variation may have been due to the excessive heat in the greenhouse accompanied with unequal ventilation.

UNIVERSITY OF WISCONSIN PEAT

Figs. 4, 5, and 6 and tables III and X show results obtained with the University of Wisconsin peat supplemented with shale and KCl. The corn and the clover in table III were grown on virgin peat, but the tomato plants of table X were grown, without further fertilization, on the same soil and in the same vessels that had grown the corn. The corn made rather consistent gains in weight and in the percentage of potassium when grown with potash shale. The responses in clover were almost proportional to the amounts of shale used. Gypsum caused enormous increases in the clover in rows no. 1b and no. 6b compared with companion rows which received similar treatment but no gypsum. This indicates that the University of Wisconsin peat is deficient in sulphur for clover. The control rows no.

1b (table III), in both clover and corn, contain a lower percentage of potassium than the companion rows without gypsum. With clover, the deficiency of sulphur in the peat and the addition of gypsum caused a great increase in growth in spite of the lack of potassium. The increase in dry weight with the limited intake of potassium decreased the percentage of potassium. In no. 4b also, of the clover,



FIGS. 1–3

the potassium content was lower than in the control. This was grown on raw shale. Later in this paper it is shown that roasting shale increases the availability of the potassium that it contains. This accounts in part for the lower percentage of potassium in plants grown with raw shale. The equally good growth of clover on roasted and unroasted shale may be due to a slight amount of sulphur in the

shale. Analyses by the Georgia State Department of Geology show that the shale contains 0–1 per cent sulphur.

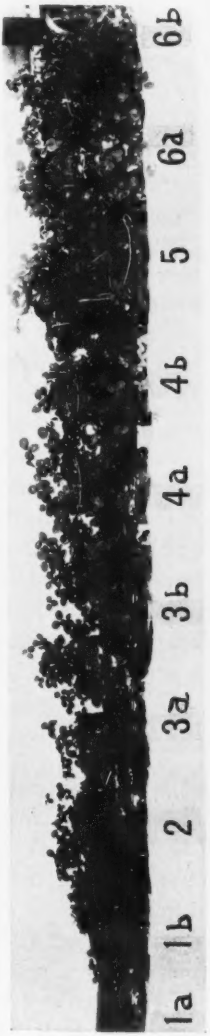


FIG. 4

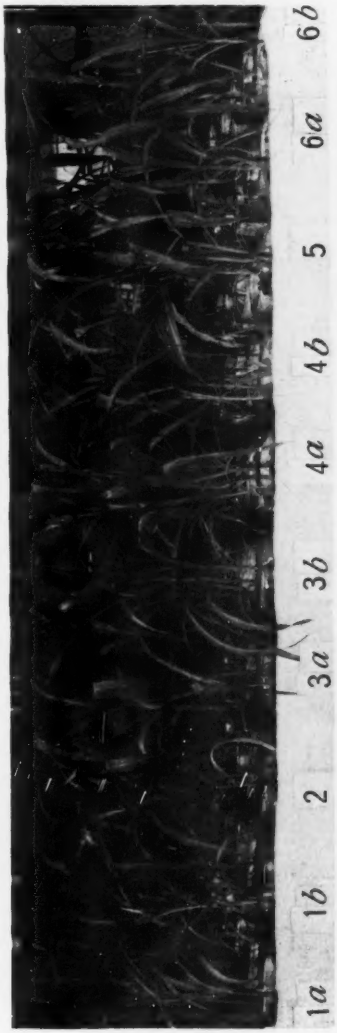


FIG. 5

Rows no. 3a and 3b test the effect of the presence of gypsum on the availability of the potassium in the shale. There is little effect seen either in growth or percentage of potassium in the tissues of the corn, but there is a doubling of the growth and a 25 per cent increase in the percentage of potassium in the clover. This gain in growth in the clover was probably due to the furnishing of sulphur, but the rise in potassium content may be attributed to an increased availability of the potassium, due to the $CaSO_4$.

FIG. 6

In rows no. 4a and 4b a test is made of the relative availability of the potassium in roasted and in raw shale. Very little difference is manifest in the growth of the clover, but the corn exhibits about 60 per cent greater increase of growth, and the potassium percentage is about 50 per cent higher in both kinds of plants, indicating a much greater availability of the potassium in the roasted shale.

CODDINGTON PEAT

The results with buckwheat and oats grown in Coddington peat are shown in table IV and in figs. 7 and 8. Each row of four pots in this experiment contained two pots treated with 2 tons of chemically pure precipitated $CaCO_3$ per acre and two pots untreated. The results with

$CaCO_3$ are shown in lines in which the numbers are followed by "L." No gains over the control are recorded for oats in pots treated with both $CaCO_3$ and shale or KCl, but five out of seven groups receiving some form of potassium without lime carbonate show increases in weight. The gains with shale vary from 0.4 to 38.2 per cent with an average of 20.6 per cent. All five applications of shale to buckwheat, except one, show benefit in dry weight,

TABLE III

PLANTS GROWN IN PEAT FROM UNIVERSITY OF WISCONSIN MARSH; INCREASED GROWTH GIVEN AS PERCENTAGE GAIN OVER CONTROL

No.	Tons of shale	$CaSO_4$ tons	Corn*			Medium red clover†		
			Growth		K (percentage in shoots)	Growth		K (percentage in shoots)
			Green	Dry		Green	Dry	
1a	0.0 control	0.0	.....	.....	1.97	.....	.....	1.59
1b	0.0	1.3	33.6	38.8	1.30	133.2	188.5	1.05
2	0.43 roasted	0.0	20.0	20.9	1.92	38.2	39.5	1.62
3a	1.3 roasted	0.0	25.9	17.9	2.53	102.6	111.4	1.82
3b	1.3 roasted	1.3	24.0	21.9	2.35	209.5	212.4	2.28
4a	2.6 roasted	0.0	28.3	26.3	3.81‡	243.7	231.9	2.07
4b	2.6 raw	0.0	7.0	16.4	2.07	246.4	253.8	1.36
5	5.2 roasted	0.0	63.3	63.7	3.07	529.0	526.2	2.60
6a	218.0 lb. KCl	0.0	71.7	68.9	5.98	402.1	380.9	4.60
6b	218.0 lb. KCl	1.3	70.5	57.3	6.29	527.2	524.8	4.45

* The corn grew 11 weeks and the clover 23 weeks.

† The peat in which the clover grew was treated with 2.5 tons of Missouri limestone per acre.

‡ Probably too high; the tissue was disintegrated by mold.

and even this exception shows benefit in green weight. The percentages of increased growth range from 0 to 30.9, with an average of 17. In all the shale-treated rows the gains were about 50 per cent better in the unlimed part of each row. Thus lime injured this peat for both oats and buckwheat. In rows 5 and 7 may be compared the effects of roasted and raw shale. In the absence of lime carbonate, roasted shale produced a gain of 30 per cent as compared with no gain on raw shale, and a slight increase in the percentage of potassium. In the presence of $CaCO_3$, however, roasting nearly trebled the increase in weight and raised the percentage of potassium about half. Roasting the shale with gypsum seemed to depress the growth of buckwheat in the unlimed pots in row 4, and to have no effect in

the limed pots, but it increased the percentage of potassium nearly half in both limed and unlimed pots. Roasting the shale alone, and with gypsum, had no effect on the growth of oats. Numbers 4 and 5

TABLE IV

PLANTS GROWN IN PEAT FROM CODDINGTON EXPERIMENT FARM; GROWTH SHOWN AS PERCENTAGES OF GAIN OVER CONTROL; K GIVEN IN PERCENTAGE OF TISSUE

No.	Tons per acre			Buckwheat			Oats		
				Growth		Potassium intake	Growth		Potassium intake
	Shale	Gypsum	$CaCO_3$	Green	Dry		Green	Dry	
1.....	0.0	0.0	0.0	Control	.....	0.94	Control	.....	1.41*
1L.....	0.0	0.0	2.0	1.6	− 0.4‡	lost	−28.6	−28.5	0.99*
1b.....	0.0	0.5	0.0	− 0.42	0.9	1.16	7.9	.....	.....
1bL....	0.0	0.5	2.0	− 0.42	− 1.1	0.91	−21.5	.....	.....
3.....	1.3r†	0.0	0.0	13.7	16.5	1.13	38.2	38.5	.....
3L.....	1.3r	0.0	2.0	− 0.03	4.2	0.96	− 4.9	−11.1	.....
4.....	2.6R1	0.5	0.0	24.4	19.3	2.83	25.6	.....	2.45*
4L.....	2.6R1	0.5	2.0	19.1	16.3	2.36	−21.3	.....	2.31*
5.....	2.6Ro	0.0	0.0	40.2	30.9	1.92	0.4	.....	.....
5L.....	2.6Ro	0.0	2.0	18.8	16.1	1.75	−21.8	.....	.....
6.....	2.6R2	0.0	0.3	33.2	24.0	2.19	−27.6	.....	.....
6L.....	2.6R2	0.0	2.3	24.0	20.6	1.91	−18.0	.....	.....
7.....	2.6 r	0.0	0.0	14.9	− 1.1	1.87	24.1	23.7	2.30
7L.....	2.6 r	0.0	2.0	9.0	6.0	1.15	−14.2	−12.6	.....
8.....	200. lb. KCl	0.0	0.0	11.0	0.6	3.48	14.9	9.3	4.67
8L.....	200. lb. KCl	0.0	2.0	27.0	10.6	2.40	− 9.7	−12.9	3.73
8b.....	200. lb. KCl	0.5	0.0	19.7	8.3	2.32	− 0.6	.....	.....
8bL....	200. lb. KCl	0.5	2.0	19.0	3.8	2.93	−45.2	.....	.....

* Because amount of tissue was so small, No. 1 was preserved and analyzed with 1b, 1L with 1bL, 4 with 5, and 4L with 5L and the potassium content is given for these pairs together.

† r indicates raw shale; Ro indicates roasted at red heat for ½ hour. R1 indicates roasted for ½ hour at red heat with 0.5 tons $CaSO_4$. R2 indicates roasted at red heat for ½ hour with 0.3 tons $CaCO_3$; L following a number indicates the part of the corresponding row treated with 2 tons of $CaCO_3$ per acre.

‡ A minus sign indicates less than control (applies to all the tables).

were preserved together because of the small amount of tissue in the oats, so no comparison can be made between roasting alone and roasting with gypsum as to content of potassium. However, the

percentage of potassium in the plants from the pots containing roasted shale is a little higher than in those from pots containing raw shale. Row 6 had the shale roasted with 11.5 per cent of its weight of $CaCO_3$. Considering both limed and unlimed pots, the effect on growth of buckwheat is about the same as when the shale is roasted alone; but the potassium intake is about 10 per cent

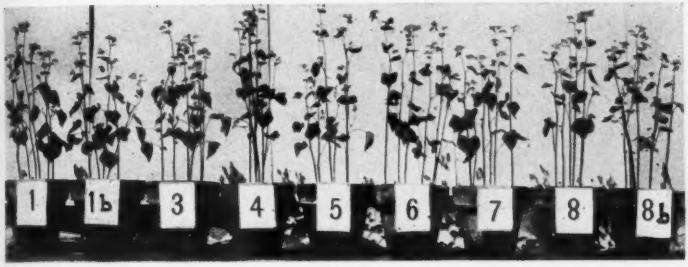


FIG. 7

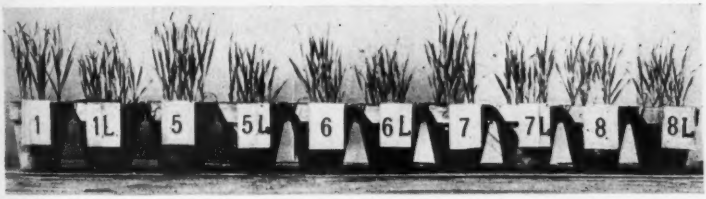


FIG. 8

higher. The oats were very sensitive to lime in this peat and were injured in the pots containing shale roasted with lime.

PLAINFIELD SAND

The results with alfalfa and winter wheat grown in Plainfield sand are shown in table V. Although not large, there is a rather consistent increase in dry weight due to shale in both of these sets of plants. Liming was beneficial in the crocks containing the wheat. Roasting the shale alone or with $CaCO_3$ or $CaSO_4$ seemed to have no effect on the growth of either set.

Hazel Crest sand

Table VI gives the results in Hazel Crest sand with alfalfa and clover. The alfalfa was benefited in only two rows out of six receiving shale, the best one gaining 17.2 per cent while the row grown on KCl increased 25.4 per cent. This clover grew in the same soil and pots as the alfalfa without further addition of fertilizers. It made rather large and consistent increases due to shale and KCl. This is

TABLE V

ALFALFA AND WINTER WHEAT GROWN IN PLAINFIELD SAND*; INCREASED GROWTH GIVEN AS PERCENTAGE OF GAIN OVER CONTROL

No.	KCl (lb.)	Shale (tons per acre)	$CaSO_4$ (tons per acre)	Alfalfa†		Winter wheat‡			
						Green		Dry	
				Green (percentage)	Dry (percentage)	No. Ca.	2.T.Ca CO_3	No. Ca.	2.T.Ca CO_3
1	0.0	0.0	0.0	Control	.....	.....	11.7	.....	0.003
2	0.0	0.0	0.52	11.1	10.1	.....	.....	.....	.....
3	0.0	2.6 raw	0.0	13.4	11.3	9.6	16.6	24.4	20.6
4	0.0	2.6 roasted	0.0	10.6	7.7	1.6	35.2	1.9	18.0
5	0.0	2.6§	0.0	12.4	11.4	.....	.....	.....	.....
6	0.0	2.6‖	0.52	12.6	12.3	.....	.....	.....	.....
7	400.0	0.0	0.0	28.2	19.2	43.6	67.4	14.2	30.9
8	400.0	0.0	0.52	28.2	17.7	.....	.....	.....	.....

* Soil treated with 300 pounds of $Ca(H_2PO_4)_2$ per acre.

† All cans for alfalfa limed with 2 tons of Missouri limestone per acre, while one-half of crocks containing wheat treated similarly.

‡ All of the wheat crocks were given 200 lb. of $CaNO_3 . 4\ H_2O$ per acre.

§ Roasted with 15 per cent $CaCO_3$.

‖ Roasted with 20 per cent $CaSO_4$.

especially striking when one considers that it was obtained with a second set of plants on the same soil without any further addition of fertilizer after the first crop was removed. Using shale roasted alone and roasted with $CaCO_3$, both seemed to cause a little better growth than raw shale. Autoclaving and roasting the shale with $CaSO_4$ and autoclaving with $CaCO_3$ did not give any benefit over raw shale.

Very unexpected results were obtained in Hazel Crest sand with corn. When first tested for deficiency of potassium by growing buckwheat in it, a good response to potassium seemed to be obtained. But with corn (table VII and fig. 9) an enormously better growth was obtained (row 1) without potassium fertilizer and with only 2

tons of $CaCO_3$ per acre, as compared with even 400 pounds of KCl and 4 tons of $CaCO_3$ per acre. The potassium in this row 1 is only about 33 per cent of that in the control and 16 per cent of that in the best growth in KCl, while the weight of tops is nearly five times that of the control and more than twice that of the best growth in potassium chloride. This must indicate that there is sufficient available potassium in the Hazel Crest sand for corn, at least up to the

TABLE VI

ALFALFA AND CLOVER GROWN IN HAZEL CREST SAND; SOIL TREATED WITH 400 POUNDS OF $Ca(H_2PO_4)_2$, 4 TONS OF MISSOURI LIMESTONE, AND 416 POUNDS OF $Ca(NO_3)_2$ PER ACRE; INCREASED GROWTH GIVEN AS PERCENTAGE OF GAIN OVER CONTROL

No.	KCl (lb.)	POTASH SHALE (TONS PER ACRE)	$CaSO_4$ T. PER ACRE	ALFALFA* (GREEN TOPS)	MEDIUM RED CLOVER†	
					Green tops	Dry tops
1.....	0	0.0	0.00	Control	.....	.....
2.....	0	0.0	0.52	− 0.5	12.2	7.2
3.....	0	2.6 raw	0.00	− 5.3	25.3	29.1
4.....	0	2.6 roasted red heat ½ hr.	0.00	− 3.8	32.5	37.8
5.....	0	2.6 roasted with 0.52 T.$CaCO_3$	0.00	− 0.5	40.5	38.9
6.....	0	2.6 autoclaved‡ with 0.52 T.$CaCO_3$	0.00	12.9	29.7	23.4
7.....	0	2.6 roasted with $CaSO_4$	0.52	1.9	17.2	15
8.....	0	2.6 autoclaved‡ with 0.52 T.$CaSO_4$	0.52	17.2	16.9	14.9
9.....	400	0.0	0.00	25.4	83.8	66.0
10.....	400	0.0	0.52	16.3	65.2	48.3

* Alfalfa grew 17 weeks and clover 19 weeks, from January to May.

† Grown in same soil and cans as alfalfa of previous year, without addition of fertilizer.

‡ Autoclaved at 15 lb. steam pressure 30 minutes.

age attained in this experiment. Precipitated $CaCO_3$ must be injurious for corn in this soil when used at the rate of 4 tons per acre. The benefits seemingly due to potassium fertilizers in this experiment must have been due to their corrective effects on lime injury. The carbohydrate-nitrogen ratio, where there was no lime injury, is seen to be about twice that of the best row injured by lime.

Discussion

BENEFITS OF RAW SHALE

From the foregoing tables it is seen that 2.6 to 3 tons of untreated shale per acre benefited most of the plants grown in potash-deficient

TABLE VII

CORN GROWN IN HAZEL CREST SAND; INCREASED GROWTH GIVEN IN PERCENTAGES OF CONTROL; K IN PERCENTAGE OF TISSUE. SOIL TREATED WITH 300 POUNDS OF $Ca(H_2PO_4)_2 . 2H_2O$ AND 416 POUNDS OF $Ca(NO_3)_2$ PER ACRE. ALL ROWS LIMED AT RATE OF 4 TONS OF CHEMICALLY PURE PRECIPITATED $CaCO_3$ PER ACRE EXCEPT NO. 1, WHICH WAS GIVEN ONLY 2 TONS

No.	Potash shale (tons per acre)	$CaSO_4$ (tons per acre)	Growth of shoots		K per cent	Nitrogen per cent				Carbohydrates				
			Green	Dry		Insol. Org.	Soluble Org.	$NH_3 + NO_3$	Tot. N	Reducing	Non-reducing	Poly-sac.	Total	Carbohydrate N
1	0.0	0.0	653.7	385.7	0.63	1.83	0.32	0.49	2.64	9.37	1.42	21.9	32.7	12.4
2	0.0	0.0	6.6	lost										
3	0.0	1.1	Control		1.75	1.73	0.72	1.81	4.26	2.04	0.35	20.9	23.3	5.47
4	3.0 raw	0.0	1.5	0.0	1.72	1.87	0.00	2.83	4.70	2.34	2.09	16.5	20.9	4.45
5	3.0 Ro*	0.0	− 5.9	6.5	1.71									
6	3.0 Ro(1)	0.0	− 26.5											
7	3.0 Auto. (1)	0.0	19.0	lost										
8	3.0 Ro(2)	1.1	66.2	51.9	1.91	1.89	1.22	1.62	4.73	2.53	3.20	16.5	22.3	4.92
9	3.0 Auto (2)	1.1	65.4	51.5	1.58	1.68	1.15	1.25	4.08	2.12	1.04	19.4	22.7	5.56
10	221.0 lb. KCl	0.0	128.7	100.0	3.46	1.65	0.93	0.90	3.48	2.17	3.17	16.5	21.7	6.24
11	221.0 lb. KCl	1.1	219.9	138.8	3.36	1.83	0.95	0.78	3.56	4.57	1.01	18.3	23.9	6.72
12	256.0 lb. K_2SO_4	0.0	114.7	80.8	3.70	2.05	0.98	1.10	3.13	1.44	0.14	19.3	20.9	6.67
13	300. lb. KNO_3	1.1	39.3	− 0.3	4.95	2.64	1.51	0.63	4.78	3.16	2.24	15.6	21.0	4.39

* Ro indicates roasted at red heat for 30 minutes; Ro(1) indicates roasted with 20 per cent $CaCO_3$; Auto (1) indicates autoclaved at 15 lb. steam pressure for 30 minutes with 20 per cent $CaCO_3$; Ro(2) indicates roasted with 1.1 ton of $CaSO_4$; Auto (2) indicates autoclaved with 1.1 tons of $CaSO_4$.

soils. These increases varied from 7 to 25 per cent. The seeming exceptions were in the Hazel Crest sand which apparently had sufficient available potassium for corn. Analysis showed that this sand contained about seven times as much potassium as the University of Wisconsin peat and two and a half times as much as the Plainfield sand. Clover made a gain of 253 per cent, but some of this increased growth may have been due to a slight amount of sulphur in the shale. Of four sets of plants analyzed for potassium and grown on potash-deficient soils, three showed the following increases in percentages of potassium due to raw shale: corn 5 per cent, oats 63 per cent, and buckwheat 100 per cent. In general the gains were larger on the

FIG. 9

peats than on the sandy soils. This may have been due to the peats being more deficient in available potash, for they contained a lower percentage of potassium than the sandy soils used (table I).

ROASTED SHALE

Tomatoes and corn made the large increases of 61.7 and 62.1 per cent respectively on quartz sand with roasted shale. However, raw shale was not tried in the quartz sand for comparison. Corn in the University of Wisconsin peat, buckwheat in the Coddington peat, and red clover in the Hazel Crest sand showed from 25 to 250 per cent more gain with roasted than with raw shale, while corn and clover contained approximately 50 per cent higher of potassium when grown on the roasted shale. Analysis showed that raw shale contained 0.065 per cent of water-soluble potassium while roasted shale contained 0.094 per cent, or about 45 per cent more than raw shale; hence it seems that roasting increases by nearly one-half the availability of the potassium in the shale.

GYPSUM AND SHALE

The effect of using gypsum with potash shale in quartz sand for tomatoes is shown in table VIII. While all four rows show gains, only two of them are great enough to be of significance. These plants were grown in a hot greenhouse in summer, and some of the benefit may have been due to the increased water-holding capacity of the sand caused by the finely powdered gypsum. In the University of Wisconsin peat the gypsum was merely mixed with the shale and not roasted with it. No benefit was shown in the corn, but the clover

TABLE VIII

TOMATO PLANTS GROWN IN QUARTZ SAND WITH SHALE PLUS GYPSUM; INCREASED GROWTH GIVEN AS PERCENTAGES OF GAIN OVER CORRESPONDING ROWS GROWN WITHOUT GYPSUM

No.	SOLUTION USED	SHALE (TONS PER ACRE)	$CaSO_4$ (TONS PER ACRE)	GROWTH GAIN, PERCENTAGE OVER NO $CaSO_4$
2	Shive's without K	0.5	0.5	4.7
3	Shive's without K	1.5	1.5	20.6
4	Shive's without K	3.0	3.0	2.7
5	Shive's without K	6.0	6.0	23.6

made a much larger growth due to the presence of sulphur. However, the clover also showed a 25 per cent increase in content of potassium. The tomato plants grew considerably better when shale was mixed with gypsum, but no analyses were made for potassium. With the Coddington peat, when gypsum was roasted with shale no effects were manifested in growth, but one 35, and one 50 per cent gain in potassium was found in buckwheat. The effects of the gypsum in the Plainfield sand were too small to be of significance, and the increases in growth in Hazel Crest sand, as already indicated, cannot be taken as due to available potassium. The corn plants grown in the row treated with shale roasted with gypsum, however, showed about 10 per cent higher potassium than the row treated with shale roasted alone. Analysis of the shale indicated 0.094 per cent of water-soluble potassium in the roasted shale and 0.124 per cent in that roasted with gypsum. Thus roasting with gypsum increased the content of soluble potassium about twice as much as

roasting without it. Autoclaving the shale and gypsum at 20 pounds steam pressure for 30 minutes seemed to have no effect on the availability of the potassium in the shale. From the growth results in the first three soils, from the increase in the percentage of potassium in the plants, and from the analysis of the treated shale, it seems that roasting with gypsum nearly doubles the amount of available potassium in the potash shale.

Shale and calcium carbonate

No shale that had been roasted with $CaCO_3$ was used in the quartz sand or in the University of Wisconsin peat although this was tried in the other soils. Oats were so sensitive to the lime in the Coddington peat that the small amount used with the shale caused injury. The buckwheat in this peat did not seem to be affected in growth but contained 9 and 15 per cent higher potassium content when the shale was roasted with $CaCO_3$ than when it was roasted without it. The red clover grown on Hazel Crest sand showed about 25 per cent gain in dry weight due to roasting the shale with $CaCO_3$. Analysis showed that the untreated shale contained 0.064 per cent of water-soluble potassium, the roasted shale 0.094 per cent, shale roasted with 20 per cent of its weight in $CaCO_3$ 0.105 per cent, and shale roasted with 33 per cent of its weight in $CaSO_4$ 0.124 per cent. The results indicate that roasting with $CaCO_3$ increased the availability of the potassium in the shale a little more than the roasting alone, but not as much as roasting it with gypsum. None of the results obtained, however, showed that autoclaving at 20 pounds pressure for 30 minutes has any effect on the availability of the potassium in the shale.

Sap acidity and utilization of potassium

Truog (**32**) believes that plants with neutral or nearly neutral sap have greater ability to feed on difficultly soluble forms of potassium than plants with more acid sap. It will be interesting to see if these results bear out that theory. There are three bases on which such a comparison may be made: (1) the percentage of gain over the control; (2) the percentage of potassium in the plants; and (3) the total amount of potassium taken in. To obtain plants with differ-

ent hydrogen-ion concentrations growing upon the same soil, would require the use of different kinds of plants. Different species of plants will have different potassium requirements, different rates of growth, and different degrees of response to potash fertilizers. It seems that the best means of comparison would be by ratios, obtained for each kind of plant by comparing the results on shale with the results with KCl. The ratio used in comparing growth was found by dividing the percentage of gain over the control obtained on shale

TABLE IX

COMPARISON OF ABILITY OF PLANTS TO OBTAIN POTASSIUM FROM RAW SHALE

PLANT	SOIL	PERCENTAGE GAIN, 2.6 T. SHALE	PERCENTAGE GAIN ON KCl	RATIO OF GAINS	RATIO OF PERCENTAGE OF K	RATIO TOTAL K	PERCENTAGE K*	SAP pH
Corn.....	U. of Wis. peat	16.4	68.0	0.25	0.35	0.25	2.07	5.19–5.52
Clover.....	U. of Wis. peat	253.8	380.9	0.67	0.30	0.22	1.36	5.6 –6.19
Buckwheat...	Coddington peat	14.9	19.4	0.77	0.53	0.53	1.87	4.18–5.00
Oats.....	Coddington peat	24.1	14.9	1.62	0.49	0.56	2.03	5.58–5.70
Winter wheat	Plainfield sand	16.6	67.4	0.25	No analyses	No analyses	No analyses	6.12–6.33
Alfalfa.....	Plainfield sand	13.4	28.2	0.48	No analyses	No analyses	No analyses	6.19–8.25

* In plants grown on shale.

by that secured with KCl for the same kind of plant grown under otherwise comparable conditions. The other two ratios were computed similarly for each kind of plant used. Table IX presents these ratios for three pairs of rows of plants. The two kinds of plants in each pair were grown on the same soil but have different sap acidities. Each pair was grown on a different soil. The sap acidities given were taken from different investigators and give the maximum range recorded for each variety of plant. The gains in tissue weight and in the percentages of potassium for the shale are based upon plants from the rows of untreated shale, because these were found to contain the least quantity of available potassium, and we are at-

tempting to compare the ability of the plants to take up potassium from a difficultly soluble source. The ratios for the percentages of potassium, and for the totals of potassium, are so nearly alike in the pairs that they do not seem to indicate any relation between the amount of potassium taken up and the acidity of the sap. However, the ratios between the gains on shale and the gains on the soluble potassium salt are approximately double in every case with plants having the more nearly neutral sap. This seems to agree with TRUOG's theory only in part, for the nearly neutral sap plants take up no more potassium from the shale than those with the more acid sap, but make better use of what they get, that is, they thrive better on the limited supply.

LIME INJURY

It has long been known that certain peat and muck soils are injured by excessive liming. LOEHWING (**16**) thinks that it is due in part to the depression of the intake of potassium in the presence of the lime, and in part to the precipitation of iron in the tissues (**17**). Two of the soils used in these experiments were injured by excess of $CaCO_3$, the Coddington peat and the Hazel Crest sand. Sandy soils which are injured by lime are very rare, but this one with a loss on ignition of only 7.1 per cent exhibits the phenomenon usually attributed to mucks and peats. In the Coddington peat (table IV) the oats average 23 per cent higher and the buckwheat 33 per cent higher in potassium where no lime was used than in the limed pots; so apparently lime restricts the amount of potassium taken up by the plants. On the other hand, some of the pots showing reduced growth in the presence of lime have a larger percentage of potassium than others making a much better growth without lime. For example, in the corn grown in Hazel Crest sand (table VII), the row given 2 tons of $CaCO_3$ to the acre produced nearly five times the weight of dry tops that was grown by the row receiving 4 tons. The uninjured row contained only 0.63 per cent of potassium while the injured row contained 1.74 per cent, or nearly three times as much. Evidently it was some other cause than potassium deficiency that produced the lime injury in both of these soils.

The percentage of ammonia plus nitrate nitrogen in the rows of corn injured by the lime is from two to four times as great as it is in

the uninjured row, while the carbohydrate-nitrogen ratio is only about one-half as large in most of the injured rows as it is in the unharmed row. This agrees with LOEHWING'S results in mucks and peats already referred to, and it may indicate, as he suggests, a reduction of carbohydrate synthesis, which in turn checks nitrate assimilation. BOBKOW and others (5) found some uncultivated soils, especially light ones, injured by excessive liming. They report that a pH 8 was produced, together with large quantities of ammonia, nitrites, and nitrates. They say that the injury was removed when the ammonia was nitrified.

RESIDUAL EFFECTS OF SHALE

Three series of plants were grown in pots of soil from which a crop had been removed and to which no other fertilizer had been added.

TABLE X

TOMATO PLANTS GROWN IN SAME PEAT AS FOR CORN IN TABLE III, WITHOUT FURTHER ADDITION OF FERTILIZER

No.	FERTILIZER PER ACRE			GAIN OVER CONTROL (PERCENTAGE)	
	Shale (tons)	KCl (pounds)	$CaSO_4$ (tons)	Green	Dry
1a	0.0	0.0	0.0	Control	
1b	0.0	0.0	1.3	− 8.0	− 14.1
2	0.43	0.0	0.0	− 10.8	− 30.6
3a	1.3 R*	0.0	0.0	3.0	− 11.7
3b	1.3 R	0.0	1.3	23.5	5.9
4a	2.6 R	0.0	0.0	18.2	8.2
4b	2.6 raw	0.0	0.0	13.4	14.9
5	5.2 R	0.0	0.0	82.9	81.5
6a	0.0	218.0	0.0	217.7	164.7
6b	0.0	218.0	1.3	164.1	105.7

* R indicates roasting at red heat for 30 minutes.

The first crops had not grown long enough thoroughly to test the residual effects of the fertilizers, but some indications of these effects are seen. The tomato plants of table X followed corn which had grown 11 weeks. The tomatoes made no gains over the control in the lowest two applications of the shale. Two other rows showed rather low increases in weights, suggesting that perhaps most of the available potassium in this soil had been taken up by the corn. However, the highest applications of shale (5.2 tons) and the 218 pounds of KCl

per acre produced about 50 per cent greater gains on the shale and more than twice the increases with KCl than was obtained with the tomatoes receiving original fertilizer treatments in the quartz sand. This must indicate that sufficient potassium remained in the rows of highest shale application and in those receiving the KCl, but not in the other rows. Soy beans were grown on this same soil after the tomato plants had grown 12 weeks and had been removed. They showed an increase of 17.1 per cent on the KCl row. With the 5.2 tons of shale no benefit was shown, probably because of wilt injury that was not visible on the other rows. Five out of six of the remaining rows made gains of 5–10.6 per cent.

The red clover (table VI) was grown on Hazel Crest soil after alfalfa without further fertilizers. It showed much better responses to the potassium fertilizers than did the alfalfa that preceded it. This better growth may have been due to two things. In the first place, the alfalfa may have been injured by excess liming and this effect may have disappeared during the interval between the crops. The soil stood in a dry place about 8 months before it was used the second time. The second reason may be that red clover responds more vigorously to potash fertilizer than does alfalfa. While the residual effect on the clover seems high, this response in the lower applications of shale with tomatoes, and with all of the shale-treated rows of soy beans, is somewhat meager. In all cases the residual effect of the KCl far exceeded that of the shale, in spite of the fact that 2.6 tons of shale to the acre added approximately twice as much potassium to the soil as did the KCl used. This seems to indicate that after the more available potassium in the shale has been used by the plants, the remainder becomes available very slowly under the conditions of the experiment. Possibly under field conditions it might become available more rapidly. After this shale had been heated to white fuming temperature with concentrated sulphuric acid for 30 minutes, only 12.4 per cent of the potassium in it was water-soluble, while 62 per cent in the shales from Union County, Illinois, and 60 per cent of the potassium in the Decorah shale of Minnesota were made soluble by this treatment. The Cartersville shale evidently contains a much smaller amount of readily available potassium than do these other two shales.

Excessive absorption of potassium

Godelewski (12), studying the potassium content of plants with regard to a sufficient supply in the soil, thinks that 2 per cent in the tissues of cereals and legumes indicates an abundance in the soil and hence in the tissues. In table III, corn is seen to have as high as 6.3 per cent of potassium, while it made nearly as good growth with 3 per cent. Clover showed its best increase with 2.6 per cent of potassium in its tops, while grown with KCl it contained 4.6 per cent. Buckwheat (table IV) showed its best weights with 1.92 per cent potassium, while its maximum percentage when fertilized with KCl was 4.67. The minimum figures here are most of them higher than Godelewski's, but his analyses were made on older plants and the percentage may decline as the plants mature. These maximum figures for potassium indicate that the plants grown on soluble KCl took up 40–100 per cent more than they needed at the stage of development at which they were cut. It seems probable that a less soluble form of fertilizer, in which potassium became available only as needed by the plant, would be less wasteful of this element.

Georgia shale as fertilizer

These potash shales of Georgia are very favorably located, near the sands and peats of the coastal plain of the southeastern states, many of the soils of which are deficient in potash. Fifty per cent of our imported potash is said to be used in the four states North Carolina, South Carolina, Georgia, and Alabama (30). Turrentine (33) states:

> These shales are soft and do not increase in hardness to any extent with depth. . . . . They can be mined with ease and at low cost. . . . . The chemical character of the material does not vary after a depth of from 5 to 10 feet has been reached. . . . . The climate is favorable for open cut mining throughout the year. . . . . The strata carry an average of 8 to 9 per cent potash. . . . . Being hydrous silicates, they are decomposed much more readily than anhydrous silicates e.g. orthoclase feldspar.

From these considerations of convenient location and ease of mining and processing, from the response to potash of the plants grown with the shale in these experiments, and the increases in potassium content of the plants due to shale, it seems that Georgia shales should form a valuable fertilizer in applications of 2 tons or more

per acre. Heating with gypsum could be employed with soils needing sulphur, as some peats evidently do, and calcining with $CaCO_3$ could be practiced for soils needing lime. Crops making large responses to the potassium in the shale, such as red clover, could be used in rotation; and the sod, when turned under, would contain considerable potash rather readily available to plants which do not secure sufficient potash from the shale itself. It was hoped that alfalfa would prove a satisfactory substitute in regions where red clover does not thrive, but the results in these experiments do not indicate it. Crimson clover was tried with the Hazel Crest sand, but this soil contained so much available potassium that no decisive results were obtained from the use of the shale.

Summary and conclusions

1. Twelve kinds of plants were grown in pots in quartz sand and in four different soils with potash shale, potassium chloride, and Shive's solution as sources of potash.

2. Green and dry weights were obtained, and considered, when compared with the controls, to indicate the benefit derived by the plants from the potash shale and from the more soluble forms of potassium.

3. The tops of a part of the plants were analyzed for potassium, showing in certain cases 60–100 per cent increase in potassium content when grown with applications of 2.6 tons per acre of untreated shale; much larger increases occurred with KCl applications.

4. The use of untreated shale showed that considerable benefit is derived from it by plants grown in potash-deficient soils, especially peats.

5. Shale roasted at red heat for 30 minutes produced greater responses than untreated shale, and was found to contain 45 per cent more water-soluble potassium. When roasted with 20 per cent of $CaCO_3$ it produced a little better response than when roasted alone, and showed 12 per cent more water-soluble potassium. Autoclaving the shale with 20 per cent $CaCO_3$ at 20 pounds steam pressure for 30 minutes did not increase the amount of water-soluble potassium over that in the untreated shale. Roasting with 37 per cent gypsum for 30 minutes at red heat gave still better growth of

plants, and increased the water-soluble potassium 30 per cent more than roasting it alone. Autoclaving the shale with gypsum at 20 pounds steam pressure for 30 minutes was of no benefit.

6. Pairs of rows of different kinds of plants grown in the same soil were compared on the basis of the ratio of the gain on raw shale to the gain on KCl. The plants with more nearly neutral sap received about double the benefit from the shale that was received by plants with more acid sap.

7. One peaty soil and one sandy soil were injured by excessive applications of $CaCO_3$, but the harm did not seem to be due to the depression of the intake of potash in its presence. Corn grown in this sandy soil was analyzed for carbohydrates and nitrogen. Results showed that the carbohydrate-nitrogen ratio was nearly twice as large when there was no lime injury, as in the case of lime-injured plants. The low percentage of carbohydrates and the high percentage of ammonia plus nitrate nitrogen suggest a checking of photosynthesis and consequently of the utilization of inorganic nitrogen. The depression of photosynthesis may be related to iron immobility in the over-limed plants.

8. Residual effects obtained by growing two or three crops in the same pots of soil without additional fertilizer indicated that, after the most readily available potassium was taken up, the remainder became available very slowly under greenhouse conditions.

9. The shale was heated with concentrated H_2SO_4 to fuming temperature for 30 minutes. Then only 12.4 per cent of the potassium in the shale was water-soluble as compared with 62 per cent of the Union County shales of Illinois and 60 per cent in the Decorah shale of Minnesota. The low solubility with H_2SO_4 and the responses with plants indicate that the potassium in this shale is considerably less available than in the other two shales.

10. The plants grown on soil treated with KCl contained 1.5–2 times as much potassium as was found in other plants of the same kind, growing almost if not equally as well, and fertilized with potash shale.

11. Shale, roasted with limestone or gypsum, would probably prove a valuable fertilizer in applications of 2 tons or more per acre, especially on peaty soils and for certain crops like red clover. Plot experiments in the field, however, are needed to test it further.

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EFFECT OF MOISTURE SUPPLY ON DEVELOPMENT OF PYRUS COMMUNIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 406

ALDEN F. BARSS

(WITH ELEVEN FIGURES)

Introduction

A number of interesting investigations have been carried on in the past in an effort to discover the effects of different environmental factors upon plant development. In recent years many of these studies have dealt with plants of agricultural or horticultural importance.

Much information has been gathered through these investigations as to the effect on plant development of such factors as light, temperature, air humidity, soil acidity, and various chemical substances. Other studies attempting to discover the relation of soil moisture to plant growth have included, among other things, estimations of the total water requirements, determinations of the wilting coefficient of various soil types, and the effect of atmospheric conditions on the ratio between the number of pounds of water used and the number of pounds of dry matter produced. So too, many irrigation experiments have been carried on with fruit trees in the orchard districts of the west, limited mainly to studies of the effect of applying water by various systems and in varying amounts on the fruit itself or on the total crop produced. Most if not all of these investigations have not recorded the actual effect of the changes in water supply upon the tree itself or upon its fruiting habits.

In order to understand this fundamental phase in the larger problem of commercial irrigation, as well as to supplement general knowledge of plant behavior, an investigation was commenced in December, 1912, for the purpose of determining the effect of varying amounts of moisture upon the growth response and tissue development of the pear tree. A preliminary report (2), showing certain

gross effects of supplying different amounts of water under controlled conditions, is here supplemented with additional records, including the results of a microscopical study of the changes in the anatomy of pear shoots as influenced by water supply.

Material and methods

To avoid the inexactness of outdoor conditions the investigation was carried on in the greenhouse. Forty-eight uniform Bartlett pear trees, dwarfed on Angers quince roots, three years old from the bud, were transplanted into rich soil (half composted horse manure and half garden loam) into 12-inch pots, a layer of $\frac{1}{2}$ inch of quartz sand placed on top of the soil, and the pots set into shallow pans to prevent loss of water by percolation. These forty-eight trees were then divided into four lots of twelve each. These lots received identical treatment in every respect save one, the application of water.

All the water was measured. By applying enough water to the trees in lot L so that there was seepage in the pans in which the pots were standing, these trees were assured a continuous supply. The trees in lot F were given the same total measured amount of water as those in lot L; but were given only one-half the amount at each application and watered approximately twice as frequently. The trees in lot M received half as much water as those in lot L (or F) by watering them every second time that the trees in lot F received their quota. The remaining twelve trees, making up lot S, received the smallest amount of water possible to retain them alive.

Only during the first season was this procedure departed from, and then only to the extent that during the last three months of the growing season the trees in lot F were given much less water than those in lot L. It was also found that occasionally the watering of certain individual trees, especially in lots L and F, had to be varied somewhat in order to maintain the same general relation between the trees in the different lots. Trees which were already supplied with all the water they could use could not be forced to take in more by applying an excess.

The total water application for each tree during the first growing season of 7 months was as follows: lot L (large amount) $49\frac{1}{2}$ quarts

to each tree; lot F[1] (frequent and large) 37 quarts to each tree; lot M (medium amount) 17¾ quarts to each tree; lot S (small amount) 11 quarts to each tree.

Since soil moisture determinations were not made during the progress of the experiment, there is the probability that the actual moisture content of the soil varied among the different trees in any one lot. Between the separate lots, however, the relative amount of water would not vary a great deal, and it is thought would rather closely approximate moisture relations that could be maintained under field conditions.

The total water application for each tree during the second growing season of 8 months was: lot L, 72½ quarts; lot F, 69 quarts; lot M, 38 quarts; lot S, 15 quarts. And during the third season of 7 months: 57 quarts, 58 quarts, 30 quarts, and 7½ quarts respectively in the four lots L, F, M, and S. Each year it was possible to maintain approximately the same ratio of watering between the different lots. At the beginning of each season all trees in all lots were given a thorough watering to start them into growth.

Each spring all open blossoms were artificially pollinated by means of a camel's hair brush with pollen from a Winter Nelis tree which had been brought in to use for this purpose.

Throughout each growing season records were made from time to time of the general appearance, habit and amount of growth, bud formation, flowering and fruiting of the trees in the four lots.

Samples of the wood growth were taken from the trees at various times to study the effect of the different watering on the tissue development. Such samples for histological purposes were taken the first year: (1) at the beginning of the experiment; (2) when growth was well started; (3) one month later; (4) three months after the previous collection; and (5) while the trees were dormant just before growth started for the second season (this would be similar to no. 1 but a full year later). These wood samples of the current growth, as representative as possible of the trees from which they were taken, were cut transversely into small blocks about an eighth of an inch long, killed and fixed in formalin-alcohol; desilicified when necessary

[1] During the last 3 months of the season, lot L received 63 per cent more water than lot F.

with hydrofluoric acid; infiltrated with celloidin; sectioned; stained with safranin and Delafield's haematoxylin, and mounted in Canada balsam on glass slides, the system followed throughout being largely that outlined by CHAMBERLAIN (5).

No chemical analyses or microchemical tests were made.

General growth studies

As is natural with any growing trees, there were rather wide fluctuations within each lot of twelve, as also between parts of a single tree. It was not to be expected that a given treatment would result in all individuals so treated producing the same number of branches of uniform length and diameter, nor an identical number of flower clusters setting and maturing the same number of fruits of uniform size. The removal of samples at various times had the effect of pruning on the remainder of that branch, with varying results depending upon the growth stage of the trees at the time the samples were taken. The bearing of fruit by certain trees in any one year and not by others also resulted in whole trees or parts of trees in a single lot giving changed responses the year following from those trees which bore no fruit. For these reasons it seems unnecessary to present full tabulations of the figures secured, but attention is drawn to the definite facts and outstanding differences which became evident during the course of the investigation.

1. FREQUENCY OF WATERING.—In no respect did the results obtained in either of the two heavily watered lots show a material difference over those of the other. Under the conditions of this particular experiment, therefore, lot L with a large amount of water and lot F with approximately the same total amount, but applied twice as frequently in half the quantity at a time, may be considered practically identical.

2. STARTING GROWTH.—The previous year's difference in watering appeared to have no influence on the time of starting growth the next season (fig. 1, upper).

3. FLOWER CLUSTERS.—Except for the first spring when there was no bloom, practically every tree in every lot produced some blossoms every year. The trees receiving the large supply of water produced during the first two years of blossoming the greatest number of

cluster buds (buds containing a cluster of flowers, leaves, and vegetative growing points within the one set of bud scales). In the second of these years, these trees exceeded those in the moderately watered

FIG. 1.—Comparison of representative trees, one from each lot, during second year of experiment; trees arranged from left to right according to water supplied: L, large amount; F, frequent and large amount; M, moderate amount; S, small amount: upper, during active growth; lower, nearing end of growing season.

lot by having almost double the number of blossoms to each tree. In the third year the situation was reversed, however, lot M trees producing almost twice as many cluster buds to each tree as those in lot L or lot F, which lots the season previous matured the greatest number of fruits. The scantily watered lot at no time equaled the others in number of cluster buds.

4. FLOWERS.—The trees in lot S in its first year of bearing averaged the greatest number of flowers to each cluster, due to the fact that a high percentage of its clusters were borne on spurs (79 per cent), whereas the heavily watered lots had almost half their clusters (45 per cent) borne laterally on the previous season's growth; such axillary clusters average fewer flowers to each bud than do spur or terminal clusters. Lot M averaged between the other two in this respect.

5. SETTING OF FRUIT.—No effect on fruit setting seemed attributable to the watering except in lot S, with least water, where the first year showed 100 per cent fall of flowers, no fruit maturing, and a very low percentage maturing the next year. In the other lots the fruit setting seemed to be largely a matter of individual tree performance. A heavy set of fruit one year was followed by a light se or none the next year.

Abscission of such fruits as did not mature was noticeably different in the lot given the least water as compared with that in the others. In the former the fruits, a dull, grayish-green color, remained small and were limp during a large part of the day, hanging shriveled for several days before finally separating from the tree. In the other lots the fruits which fell were of good size and to all appearance fertilization and early development had been normal, with a full quota of plump seeds forming in most specimens examined. The dropping of these fruits came as a distinct cutting off or separation preceded by a pronounced yellowing at the point of attachment between stem and cluster base, the fruits falling of their own weight, while still a clear green and turgid.

6. MATURED FRUITS.—The number of fruits matured, especially where more than one was borne on a single tree, seemed to have had a greater effect on the size of the individual fruits and on the crop the following year than did the actual amount of water (table I).

It is evident that with a small crop even the trees receiving a medium amount of water were able to produce full-sized fruits, averaging much larger than those in the heavily watered lots, which were maturing more fruits that year. When the size of crops borne in these lots was reversed, as in the first year, the average size of fruit was reversed (figs. 2–7). It is worthy of note in this connection, however, that in the year of this large crop, the trees given a moderate amount of water produced much smaller fruits on the average than did the heavily watered trees in the year of their large crop. Watering, then, pronouncedly influenced the size of the fruits under the conditions of this experiment.

TABLE I

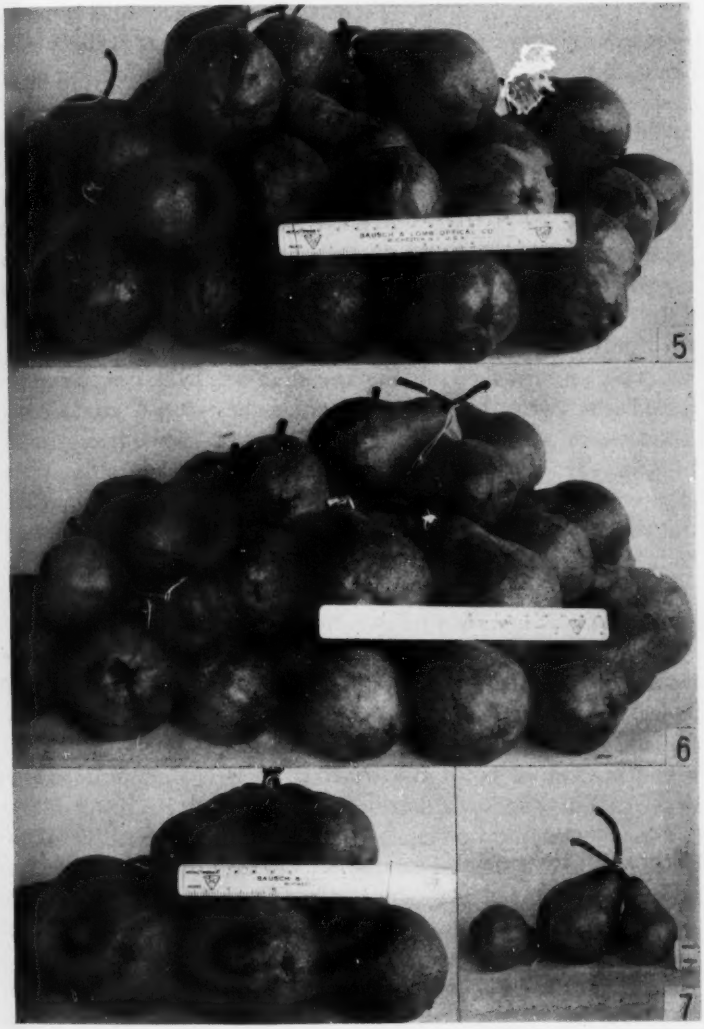
FRUIT RECORD

LOT	PERCENTAGE CLUSTERS MATURING FRUIT		NO. OF TREES MATURING FRUITS		NO. OF FRUITS MATURED		AVERAGE WEIGHT OF EACH FRUIT (GM.)		AVERAGE SPECIFIC GRAVITY	
	Year		Year		Year		Year		Year	
	1	2	1	2	1	2	1	2	1	2
L	16.0	18.0	6	10	12	50	202.6	138.8	0.9942	0.984
F	9.3	18.3	4	10	7	49	236.9	147.4	0.9958	0.987
M	33.3	6.4	7	7	20	9	93.5	174.4	1.0411	0.9915
S	.....	4.5	.....	2	.....	3	.....	41.6	.....	1.08

For the first crop, it may be said that at the time of harvesting and when ripe, the fruits in the lot given a medium amount of water differed from those in the heavily watered lots by being not only smaller, but showing less the characteristic uneven ribbing of the surface. The color was at first a dull bluish-green instead of a clear lively green, which later, however, changed to the normal yellow on ripening. They also lacked the crisp firm feel to the touch. When ripe these fruits in lot M had a tough skin and flesh, and such poor flavor and quality as to make the fruit decidedly undesirable for eating. The following year, however, lot M, with a small crop as compared with lots L and F, bore fruit of the usual high quality normal for the Bartlett variety, just as did lots L and F with many more fruits maturing. The fruits in lot S were small, poor flavored,

gritty and astringent even when ripe. The watering noticeably affected not only the size of the fruit, but their flavor and quality, especially in a large crop year when the water supply was limited.

FIGS. 2–4.—Crop produced first fruiting year under varying amounts of water: upper (lot L), large amount; center (lot F), frequent and large amount; lower (lot M), moderate amount.



FIGS. 5–7.—Crop produced second fruiting year, under varying amounts of water: upper (lot L), large amount; center (lot F), frequent and large amount; lower left (lot M), moderate amount; lower right (lot S), small amount.

7. VEGETATIVE GROWTH.—Probably the most noticeable variation in response to the application of different amounts of water was in the nature and amount of vegetative development. Considering as a shoot any new growth of the current year 4 cm. or over, it was found each year that the heavier the watering the greater were these shoots in number, length, average diameter, number of nodes, number of leaves to each shoot, and average size of leaves. The length of the growing season was also much greater. The scantily watered trees were lowest in all these features, the moderately watered trees ranging midway between the extremes (fig. 1, lower).

In the heavily watered lots the first growth in each season was frequently followed by a second or even a third growing with little cessation between the growths. The other lots had a much shorter period of first growth, however, and where any second growth came it followed a period of several weeks during which the trees seemed to have gone into a state of partial dormancy. In the fruiting years a renewed period of vegetative activity followed harvesting of the fruit, such new growth being limited largely to the trees, branches, and cluster bases from which the fruits had been picked. Again such response came occasionally from the bud just below the place from which a branch had been removed for histological study.

An indication of relative tree size is shown in the average circumference of the trunk taken at the end of three seasons of growth under the varying treatments accorded: lot L, 70.9 mm.; lot F, 68.3 mm.; lot M, 60.2 mm.; lot S, 55.5 mm.

8. RELATION OF SHOOT GROWTH TO FRUIT.—There appears to be a close qualitative and quantitative relationship between shoot growth and fruit development which seems to account for the great variations which occurred within each lot, even when all the trees in the lot had been given the same treatment. The general tendency for flower and fruit development to be greatest where shoot growth was least and vice versa was pronounced in all four lots, a correlation which has been noted and discussed by several workers in connection with their own investigations. Again it was found that the largest fruits in any one lot were generally on a tree where fewest fruits were matured, the smallest average weight to each fruit being usually where most fruits were matured.

The results secured with these trees would seem to indicate that under the conditions of this experiment, even though watering appeared to be able to alter in a measure the degree of expression as shown by number and length of shoots, number and size of fruits, nevertheless growth and fruiting appeared to have a direct interrelation over and above the effects produced by the different amounts of water applied. That is to say, by heavy watering it was not found possible to force all trees so treated into a condition of extreme vegetation with no flowering, or by medium watering into a condition of moderate vegetation and heavy fruiting, or by scanty watering produce trees which neither grew branches nor formed flowers.

A further observation may here be recorded, although its significance is uncertain, since it is realized that tree records from different seasons do not lend themselves to exact comparison.

The third growing season, although the trees in lots L and F were older and larger than they were the year before, they actually used on the average considerably less water to each tree than they did the previous year, even though at all times given as much water as they would take up. Heavy fruiting and a lower total shoot growth were associated with this lessened water consumption. These conditions might suggest that a large fruit crop makes less demand on the water supply than does a large shoot growth with its extensive leaf surface.

Histological studies

In order to make possible a direct comparison of tissue development in wood sections from the different trees at the several dates, a typical section was selected on each slide which represented a distinct branch area (near the top of the new growth, at the center, or near the base), and camera lucida drawings with uniform magnification were made of these sections. By means of some four hundred such drawings, three representative examples of which are shown in fig. 8, a careful study was made of the gross topography of the main tissues, to see the proportionate amount and placement of these tissues in the trees of the four lots L, F, M, and S.

This grosser microscopical study was followed by a more detailed microscopical examination of the separate cells as seen in cross-section; noting the proportion of the different kinds of cells existing

in the several tissues, the size of the individual cells, and the thickness of their walls in comparative regions on branches from the different lots. Figs. 9–11 show representative camera lucida drawings used in this study.

That the trees were satisfactorily uniform at the starting of the experiment was evident from an examination (from the sectioned material) of the growth of the previous year, taken at the time of transferring the trees to the greenhouse, December 28. By February 20 enough new growth had been made to provide a second set of

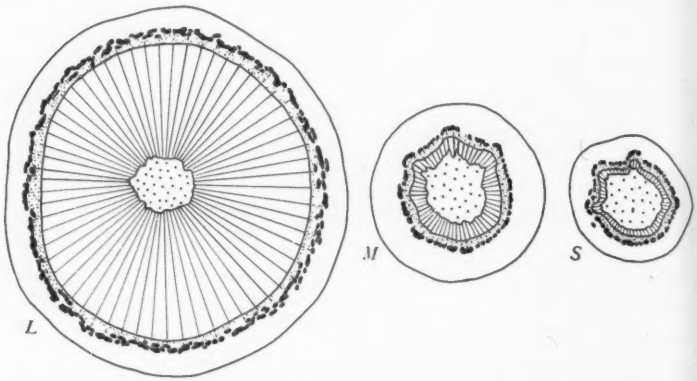


FIG. 8.—Transverse section of branch from each of three representative trees given different amounts of water, to show comparative topography of various tissues in three lots (camera lucida outlines); details of sections shown in figs. 9–11: left (lot L), large amount; center (lot M), moderate amount; right (lot S), small amount.

samples. Examination of this sectioned material indicated uniformity in this young growth. The third set of samples, taken one month after the second (on March 21), again showed no outstanding difference between the four lots. As is normal in any tree growth, there was considerable variation within each lot, apparently as great as between the four lots. Even by that date the different watering had not made its effect noticeable. The fact that the soil had been thoroughly saturated at the time the trees were brought into the greenhouse probably accounts for this continued similarity in the four lots.

The fourth set of wood samples was taken from half the trees in

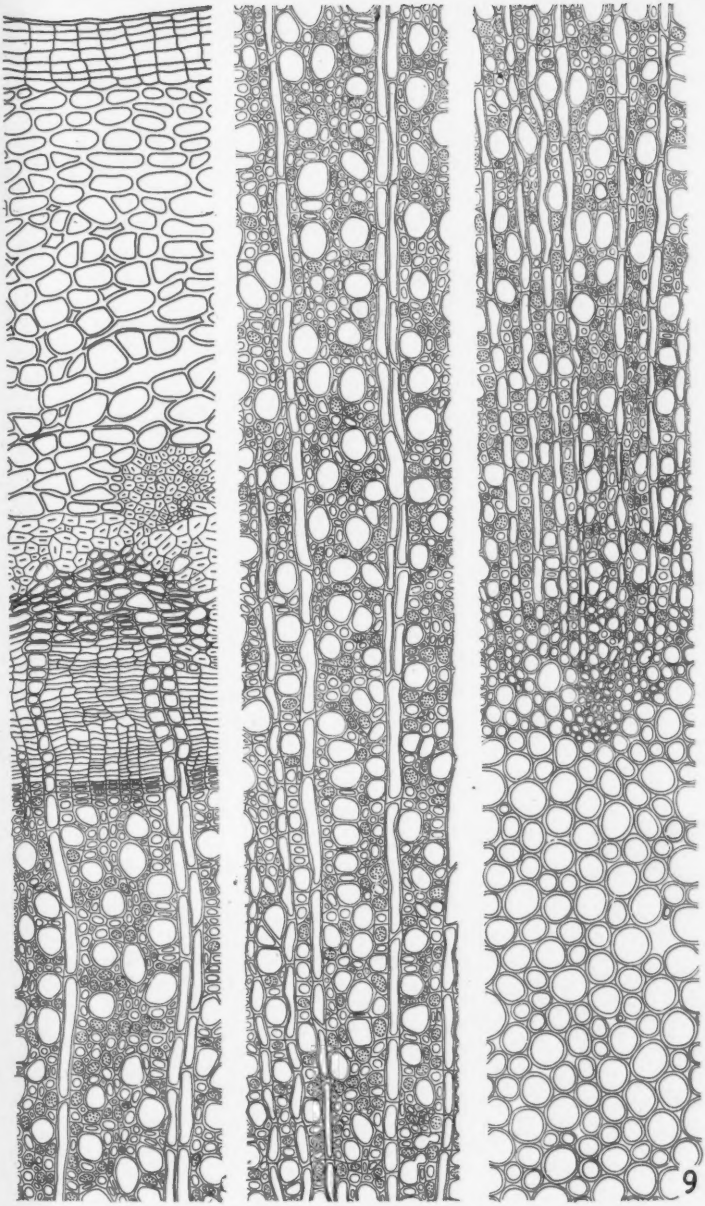
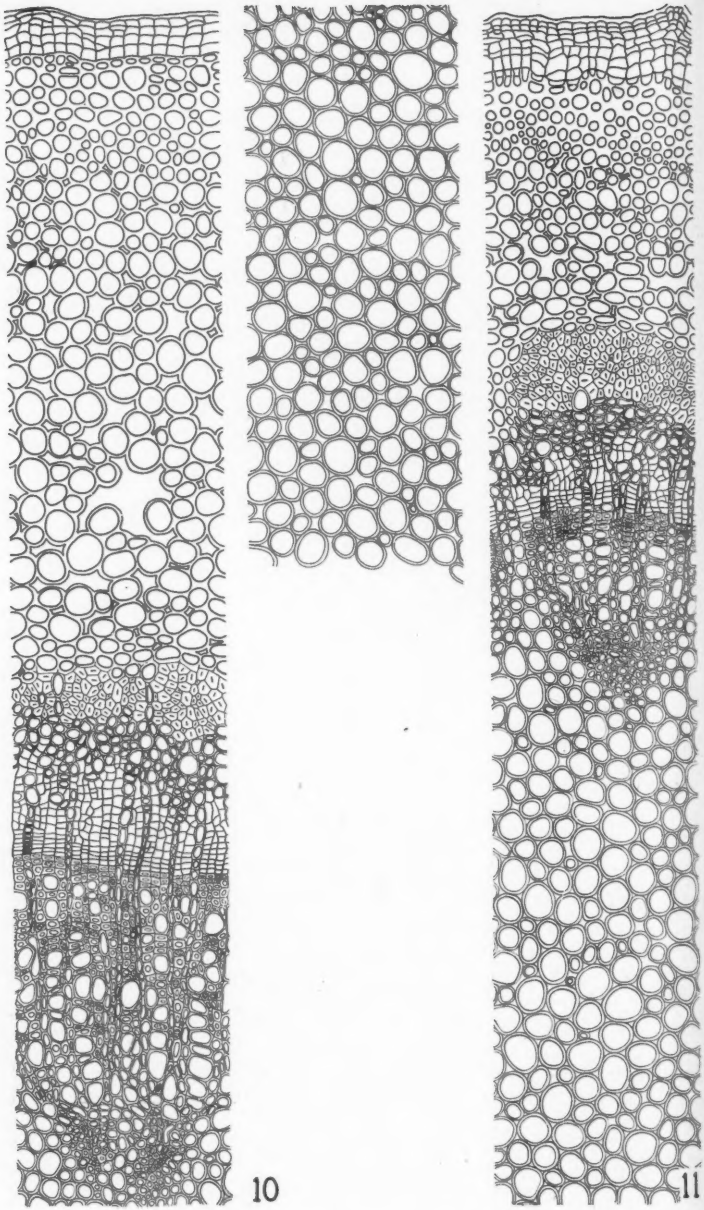


FIG. 9.—Detailed (camera lucida) drawing of narrow strip from cork to center of pith, from transverse section shown in fig. 8 L, taken from lot L (large amount); dotted cells in xylem represent parenchyma (scale same as for figs. 10, 11).



FIGS. 10, 11.—Detailed (camera lucida) drawings of narrow strips from cork to center of pith, from transverse sections shown in fig. 8 M and S (scale same as for fig. 9): left (lot M), moderate amount, cork through xylem; center (same), continued from xylem to center of pith; right (lot S), small amount, cork to center of pith.

each lot on June 16 and from the other half on July 1, or approximately three months after the previous samples had been removed. In these samples the first pronounced difference was found to hold consistently throughout a given lot of trees.

Comparing the trees in lot L with those of lot F, however, there does not seem to be any outstanding difference. Both lots show a range of variation, but not such that it is possible after a minute study of the sectioned material to say that any one thing is typical either of lot L or of lot F and is not to be found in the other. Because of this similarity, lot L only was finally selected for the detailed study of the heavily watered trees.

The fifth set of samples was taken December 27 while the trees were fully dormant, just before being started on their second season of growth under the conditions of this experiment. In this final collection for the year, one typical branch was taken from each lot. Figs. 9–11 are from this set, and bring out the contrasts between the trees in the heavily, medium, and scantily watered lots. The sample from lot L is from the first of two growths made in the one season from the one branch. The others represent but one growth.

In fig. 8 the cortex appears about the same in depth in all three lots, with the medium-watered lot showing somewhat the greatest depth. The pericyclic fibers, while more interspersed with parenchyma cells in lot L than in the other two, are well developed in all. The phloem seems not pronouncedly different in size in the three lots. The pith shows a marked uniformity in size in all lots. The xylem provides the most noticeable contrast in these contour drawings, where lot L shows a depth of xylem equal to the diameter of the entire section of the scantily watered sample. A numerical comparison of distance from cambium to pith in lots L, M, and S is 15 to 2 to 1 respectively, which if translated into terms of total area would show the heavily watered lot having an enormously greater development of xylem than either of the other two.

In the detailed drawings (figs. 9–11) of these identical cross-sections, additional facts are brought out which are shown in table II. Only the pronounced differences have been recorded. This table shows that the differences in the effect of varying water supply are not limited to the total or proportionate amount of the separate

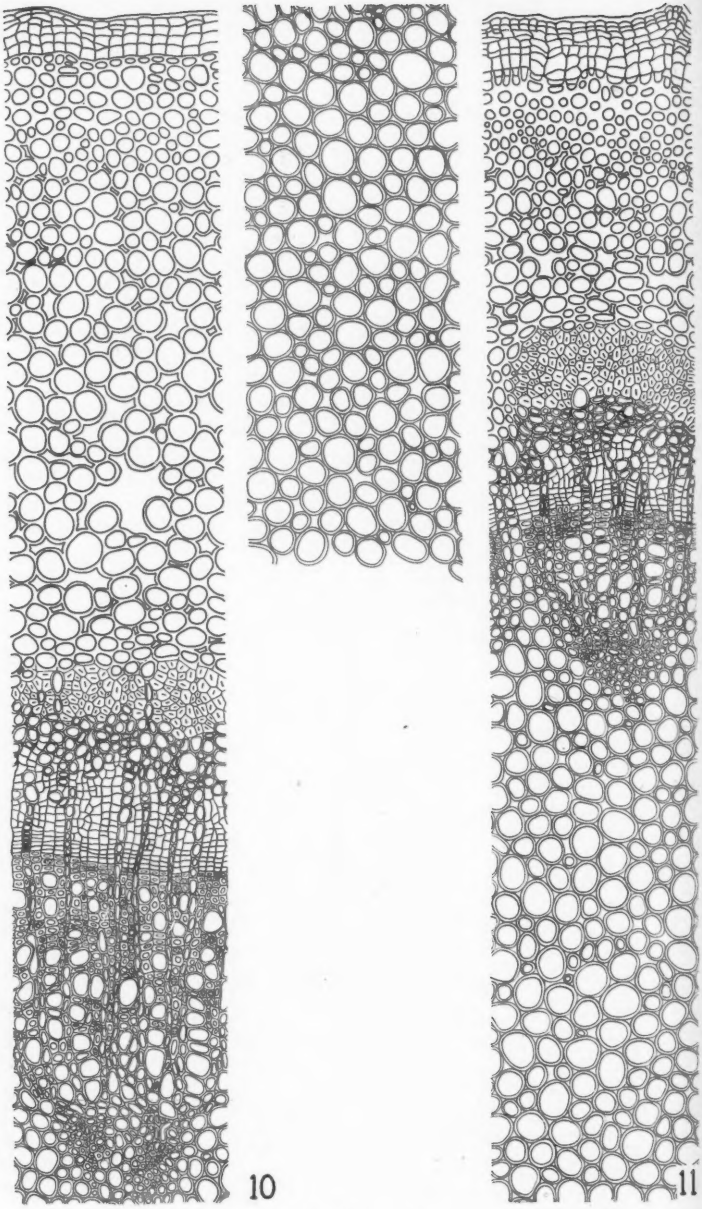


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TABLE II

CELLULAR DIFFERENCES ASSOCIATED WITH VARYING AMOUNTS OF WATER

LOT L, LARGE AMOUNT OF WATER	LOT M, MEDIUM AMOUNT OF WATER	LOT S, SMALL AMOUNT OF WATER
	Cork	
Cells large	Cells medium	Cells small, most layers
	Cortex	
Sclerenchymatous cells largest, oval in outline Parenchymatous area medium in size, smallest in rows of cells from cork to pericyclic fibers; cells large, oval	Sclerenchymatous cells medium, circular in outline Parenchymatous area large; largest in rows of cells from cork to pericyclic fibers; cells rather large, circular	Sclerenchymatous cells small, circular in outline Parenchymatous area small in size; slightly above L in rows of cells from cork to pericyclic fibers; cells small, circular
	Pericyclic fibers	
In several bands interspersed with parenchyma cells Some cells large; some stone cells present	Similar to S in cell size and depth of area	Similar to M
	Phloem	
Largest in area, largest in number and size of cells	Medium in area and in number and size of cells	Smallest in area and in number and size of cells
	Cambium	
Cells largest	Similar in M and S	Similar in M and S
	Xylem	
Enormous development; all cells largest; vessels most in number and largest in size. Ray cells longest and widest; least definite "summer" wood	Area much smaller than L, much larger than S; all cells smaller than L, slightly larger than S; largest and most definite area of "summer" wood	Area smallest in extent, cells fewest in number and smallest in size. Narrow band of definite "summer" wood. No xylem parenchyma cells other than rays
	Pith	
Size of individual cells, thickness of cell walls, and total extent of pith area seems practically uniform in all lots		

tissues, as shown in the topographical cross-section, but to the individual cells as well.

The heavily watered lot shows in its cellular composition a decided increase in the size of almost all cells, cortical, fiber, phloem, xylem; the vessels in the latter being much larger in the last-formed wood than those in the same position in the sections shown from lot M or lot S. The moderately watered lot, in the section illustrated, shows the greatest development of cortex, but in most other respects appears to take a medium position in both size and number of cells between lot L and lot S.

The scantily-watered lot has, in general, the smallest and fewest cells. It shows the greatest development of cork in number of layers, however, though not in actual depth of area. The underlying sclerenchyma seems proportionately heavier in this lot with smaller cells and thicker cell walls. All cortical cells in lots M and S appear circular in outline, whereas such cells in lot L are oval, with the longer axis in the tangential position. In phloem and xylem the cells are smaller in size and fewer in number than in the other lots.

The pith area in all lots appears to be practically uniform in size and number of individual cells, and in thickness of cell walls. This is the only feature in which there is noticeable similarity throughout, but here it is very pronounced.

The production of "summer" wood as compared with "spring" wood is most evident in lot M and least evident in lot L.

Discussion

From the data presented it is apparent that the different lots provide strong contrasts which are directly associated with the amount of water applied. In most of the gross characters, such as size of tree, number of branches, number of blossom clusters, size and number of leaves, etc., the results in general accord with the findings reported by a number of investigators, and so scarcely need reviewing in detail to emphasize the broader relation of moisture supply to plant growth. Certain other features, however, are worthy of special note.

The present experiment does not serve to illustrate the danger of over-irrigation mentioned by many writers, since even those trees

receiving the greatest amount of water, which under other conditions might prove detrimental, suffered no apparent injury from this source.

With regard to the two different systems of applying the same total amount of water, the close similarity of the results secured in lots L and F has made it impossible to conclude that either method is better than the other. The situation is probably somewhat analogous to that reported by VEIHMEYER (21), who states that provided the moisture content is above the wilting coefficient, roots apparently are able to obtain water as readily when the moisture content is low as when the soil is saturated to its maximum capacity. The fact that the soil in both lots L and F was never deficient in water may account for the uniformity of growth. The same would be true of the fruits in these two lots. With moisture present at all times considerably above the wilting coefficient, even though possibly fluctuating more in L than in F, it would seem that the fruits in both lots could develop at their maximum rate, so far as this was affected by water supply, regardless of whether that supply was maintained by a greater or lesser frequency of application.

This same situation would appear to account for the findings of LEWIS, KRAUS, and REES (15), who observed little difference in the experimental plots between 1000 and 3000 gallons per tree throughout the growing season; and for the experience of BATCHELOR (3), who found as large size and large yield of peaches with 31 acre-inches of irrigation as with 62 acre-inches under the same condition. If, as seems to be the case, the lesser amount was able to give maximum development so far as this was controllable by watering, doubling the supply would not be expected to increase the development. On the other hand, it would appear that in lot M, while reducing the seasonal amount of water to half that given the heavily watered trees evidently did not bring the water supply so low as to interfere with maximum fruit development in the year of its small crop; it did result in much smaller fruits in the year of its large crop, showing that trees of not greatly increased size may require much larger quantities of water to develop a large amount of fruit.

The results in lot M would also seem strongly to support the evidence of other investigators in favor of thinning of fruits to secure increased size in case of water shortage. In one experiment (15) it

was found that several check (unirrigated) trees when carrying a very light load produced apples which would compare favorably with those from the irrigated plots; but of another orchard it was stated, "if at least one-fourth more of the fruit had been removed at thinning time, there would likely have been not only a much larger percentage of commercial fruit but more boxes of first-class apples."

Referring to lot S, which matured no fruits the first blossoming year, and only three fruits the second year (fig. 7), this poor set as well as the small size of the fruits which did mature seem directly attributable to the water supply, either alone or as affecting the metabolism of the trees. HEINICKE (**11**) states that unfavorable conditions of nutrition and water supply are two basic factors causing the normal drop of flowers and partly developed fruits of the apple. CHANDLER (**6**) mentions that great water deficit, whether resulting from high transpiration or from limited water supply, may cause abscission of nearly all fruits. He further adds that if the water supply should be inadequate through a considerable part of the day, the leaves with their much greater osmotic concentration might pull water away from the fruits. This, under conditions of moderate shortage of water, may account for the smaller fruits.

The poor quality of the S fruits, and of the M fruits in the year of the large crop, would appear to be explained by SORAUER's (**20**) statement that with a scarcity of water there is less increase in stored food materials: acid is less, sugar is less, giving an insipid taste and poor quality.

As to the gross histological differences brought out in fig. 8, these show that the most noticeable effect associated with increased water supply is the greater depth of the xylem area. This would accord with the findings of HUNTINGTON (**12, 13**), DOUGLASS (**7, 8, 9**), MACDOUGAL (**16**), ANTEVS (**1**), and others making a study of the annual rings of forest trees in relation to climate, who report that with limited moisture, rainfall was unquestionably the controlling factor in growth as shown by width of ring. But in moist climates the trees seemed to depend on other elements, such as temperature or a combination of elements.

PEARSON (**18**), studying saplings of western yellow pine, found moisture the dominant factor, the April and May precipitation being most important in determining the amount of height growth secured.

That there was an extended period of growth associated with an increase in water in the present experiment is shown by the delay in forming terminal buds on the shoots in lot M as compared with lot S, and the much longer growing period of lot L, even to the extent of second and third growths in the one season. These observations emphasize the importance of moisture supply in connection with the laying down of new wood, when other nutrients are not lacking.

With regard to the microscopic differences as shown in table II and in figs. 9–11, the contrast in development is seen to be most pronounced. The evidence presented does not agree with that reported by CANNON (4), however, who states that the non-irrigated stems of the desert plants studied have larger ducts and more of them per equivalent cross-section than the irrigated stems. He notes, however, that VOLKENS, studying Egyptian desert plants, found contrary results, namely, that the ducts of the stems of the shrubs and trees were relatively poorly developed, which latter would appear to confirm the present evidence. Here it is shown for the pear, and seen to some extent in lot M as compared with lot S, that associated with additional water is an increase, especially in the number and size of certain types of cells, including vessels, an increase which in lot L amounts to a great development of conducting area as compared with that in the other two lots. Instead of a small amount of water in lot S causing small cells with thick walls to be formed, as PALLADIN (17) mentions, in the pear at least far fewer cells are produced under a shortage of water. Further, the cells formed are not provided with thicker walls, even though compared with the smaller size of cell they may appear so. It would seem that reducing the water supply to a minimum has not caused the plant merely to produce a given quota of cells which are smaller and thicker walled than might have been secured by providing more water, but rather, the shortage of water has resulted in stopping all cell division and enlargement beyond that which followed the first heavy watering of the year.

There is an additional point which might be of considerable practical significance to men interested in forestry, as well as to fruit growers and nurserymen. While there are instances perhaps where this is not true, in the case of the pear tree at least the great develop-

ment of xylem, accompanying increased water supply to the trees in rich soil, apparently may result not in an expanded zone of spongy, succulent tissue, but in a greatly increased volume of firm, sound wood.

The uniformity of pith in all lots is in accord with the expectations from a knowledge of plant growth. In the pear the pith is entirely a primary structure. The cells, upon being differentiated at the growing point, mature early, after which further growth ceases. In this instance, to start growth at the beginning of the season all trees were given a uniformly heavy watering. The effect of this would naturally be reflected in the primary tissues, giving uniform early growth. The reduced water supply later would appear to have made its effect evident first in the early stopping of length growth, which would result from no more cells being differentiated at the growing point. The later effect would be evident in the secondary meristem and result in reduced and finally stopped growth from the cambium.

In an attempt to account for all the gross differences noted, the results would seem to suggest a number of possible interpretations.

Recent investigators have shown the importance of the individual plant or even a part of the plant and its nutritional balance at any particular time as correlated with the type of response which may result from the treatment accorded it, whether pruning, fertilizing, shading, ringing, defoliating or what not. There is in this, then, one possible explanation for the results of the present experiment, and that is that the varied water supply, coupled with an abundant nutrient supply, modified the nutritive conditions within the trees.

In some respects there appears to be a somewhat close similarity in these four lots of trees to those of the four classes or groups suggested by KRAUS and KRAYBILL (14). Under the conditions of the experiment, the trees in lots L and F, which were given an abundance of water, gave a highly vegetative response both in external and internal characters. Also, while these trees when considered as a whole did produce blossoms and did mature fruit, such were frequently limited to certain parts of the tree, while many of the branches which made long growths failed to flower. These trees would seem to correspond to the class II trees but be closely ap-

proaching those of class III. The trees given a moderate supply of water, lot M, and those given a scanty supply, lot S, would seem to accord rather definitely with class III and class IV trees respectively.

This close similarity is of more than passing interest. At the same time there are insufficient data available to permit of more than the mere recording of the general similarity and noting its possible bearing in interpretation of the results secured. It would appear that under the conditions of this experiment a change in the amount of available soil moisture definitely caused changed expressions in tree growth, somewhat similar to those secured by other workers through changes in the nutritive balance brought about by various practices other than alterations in the water supply. Another possible explanation for the results secured is that the effect may have been largely a physical one, or a combined physical and chemical one.

Knowledge of plant growth emphasizes the importance of turgor in cell division and enlargement. The presence of an abundance of water would be expected to provide a somewhat constant condition of turgidity, and hence, other things being equal, a condition favorable for growth. Decrease of the water supply to a minimum would probably so reduce cell turgor in the meristematic regions that finally further active growth, even if nutritionally possible, would be physically impossible.

Evidently the amount of water which a tree uses does have a definite effect on the metabolic processes as shown by different expressions of growth. Where growth is checked accompanying a reduced water supply, this may be due to a single factor or to a combination of factors. It may be caused by a limiting of the supply of nitrogen, as some hold, or to an accumulation of carbohydrates. CHANDLER (6), however, contends that there "is no convincing evidence that the mere accumulation of carbohydrates inhibits growth though it might cause the nitrogen to be combined in larger organic molecules and possibly in forms less active in immediate growth." Again, the cause may be a limiting of both nitrogen and carbohydrate content, or it may be the result of alterations in the metabolism induced by lack of sufficient water to expand the tissues, the loss of turgidity being responsible for retardation and finally cessation of

development. In the present instance there is not sufficient evidence to establish proof one way or another.

It is not intended to give the impression that water of itself can bring about maximum plant development. The great importance of other factors as affecting the metabolic processes must not be lost sight of. In this connection, the relation which both the physical condition of the soil and the supply of available nutrients may bear to the water requirement of plants is of special significance.

GARDNER, BRADFORD, and HOOKER (10) state:

> Few realize that, when the soil provides conditions for tree growth that are optimum from the standpoint of nutrient supply, actually less water is required for a given yield than when the plant is handicapped because of the lack of some nutrient as well.

They also refer to LEATHER, who studying the question in India reported that

> the effect of a suitable manure in aiding the plant to economize water is the most important factor which has yet been noticed in relation to transpiration.

They also add that

> the reduction of the water requirement of the plant by maintaining the soil in a condition as near as possible to the optimum with respect to nutrient supply should be a constant and conscious aim in scientific orchard management.

This correlation between available plant food and water requirement is further emphasized by POWERS (19), working with field crops, who states

> frequently in our experiments application of a simple fertilizer has saved from a quarter to a half of the total irrigation and has doubled the returns from each unit of water.

He also reports that at times one ton of manure may equal 100 tons of water in securing returns. In an experiment with fruit trees (15) still further evidence is presented, where trees on good soil with cultivation but no irrigation were found to produce not only more but larger fruits than those on poorer soil which had an abundance of water. This serves to show not only that favorable nutritive conditions make for water economy, but that irrigation cannot be expected to make up for poor quality of soil.

Summary

1. The experiment here reported was undertaken to determine the effect of varying amounts of water upon both the gross and the microscopical development of *Pyrus communis*.

2. Forty-eight dwarf pear trees were divided into four lots, all lots being treated uniformly in all respects save for watering.

3. In the two heavily watered lots, applying the same total amount of water, but in more frequent applications of smaller quantities at a time, appeared to give results practically identical with larger applications at less frequent intervals.

4. Flower clusters were more abundant on trees given more than the minimum amount of water. The number varied between moderately and heavily watered trees, depending in part on the crop of the previous season. The number of flowers to each cluster averaged less when abundant water was supplied, due to the high percentage of axillary clusters produced, as compared with less heavily watered trees which had a high percentage of spur clusters, which latter produced proportionately more flowers to each cluster.

5. Abscission of blossoms and partly developed fruits was more pronounced where least water was available. Two types of abscission were noticed between the trees given least water and the others.

6. The number of fruits matured by a tree in any one season appeared to have a greater effect on the size of the individual fruits and on the crop produced the following year than did the actual amount of water applied to that tree. With a small crop of fruit, moderate watering was associated with production of full-sized, normal fruit. With a large crop, moderate watering was associated with production of much smaller and a poorer quality of fruit than when abundant water was applied. A pronounced shortage of water resulted in small fruits of poor flavor and quality. The value of thinning to secure increased size in case of water shortage is emphasized.

7. Abundance of water was accompanied by increase in all types of vegetative growth and in length of the growing season; scarcity of water by lowest vegetative responses; a moderate supply of water by a medium vegetative growth.

8. There appeared to be a close qualitative and quantitative relationship between shoot growth and fruit development, regardless of the amount of water that a tree received. A large fruit crop seemed to make less demand on the water supply than did large shoot growth.

9. A detailed histological study showed the most pronounced difference in gross topography of the tissues to be in the extent of the xylem area. As between a large, medium, and small amount of water, this stands in the proportion of 15 to 2 to 1 respectively. The cortex is largest in the moderately watered lot.

10. A comparison of cells showed a number of definite differences in the cortex, phloem, and xylem cells in the different lots, especially as to number and size. The pith cells seemed practically identical in all lots, regardless of the amount of water applied.

11. In the pear at least, a great development of xylem may accompany increased water supply to the trees in rich soil without resulting in succulence, but in a greatly increased volume of firm, sound wood.

12. An attempted interpretation of the results secured suggests that, following changes in the water supply, alteration in the metabolic processes may be due to physical or nutritional causes or both.

13. Emphasis is laid on the relation which the physical condition of the soil and the supply of available nutrients may bear to water requirement in securing its greatest practical efficiency.

The present delayed study of the sectioned material, prepared by the writer while connected with the Oregon State Agricultural College, has been made possible through the courtesy of Professor W. S. Brown and Dr. E. M. Harvey of that Institution. Dr. E. J. Kraus of the Department of Botany of the University of Chicago gave valuable suggestions and criticisms during the investigation.

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CYTOLOGICAL STUDY OF OEDOGONIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 407

HIRO OHASHI

(WITH PLATES I–III AND TWENTY-ONE FIGURES)

Most algae as common and as important as *Oedogonium* have been studied ever since the first microscopes were made; but while the morphology and taxonomy of this genus have been investigated by various workers, little information has been obtained concerning its cytology. Consequently it seemed worth while to make a cytological investigation of *Oedogonium*, with modern methods of technique.

Material and methods

Material used for this study, consisting of four species, was collected from four different places at various times. One new species (*O. nebraskense* Ohashi) (12) was collected by Dr. ELDA WALKER on April 22, 1919, in small ponds at Lincoln, Nebraska, and turned over to the writer. The second one, *O. americanum* Transeau, was given by Dr. G. S. BRYAN, who collected it in the vicinity of Madison, Wisconsin. The third species, *O. grande* Kuetzing Wittrock, was gathered in 1924 from a small swamp at Palos Park in the vicinity of Chicago. The fourth was an unidentified species from lagoons in Jackson Park, Chicago, collected in 1926.

In 1924 the collection of material was begun on July 3 and continued at frequent intervals until September 10; and in 1926 it was begun on May 17 and ended August 15. In both years collections were made several times at each place. Some of each collection was fixed immediately, but the greater part was kept growing in jars in a window of the laboratory.

Throughout the investigation, living material was studied both in the field and in the jars kept in the laboratory. When collections were abundant, some of the material was kept in water from the pond, and some was grown in various nutrient solutions which might vary the course of the life history.

The material collected by Dr. WALKER was fixed and preserved in a chrom-alum solution (water 100 cc., chrom alum 2 gm., formalin 1 cc.), but the remainder was fixed in chrom-acetic acid (water 100 cc., chromic acid 1 gm., acetic acid 3 cc.), with ten drops of osmic acid (1 per cent) to 100 cc. of the solution.

The zoospores and the sporelings were wrapped in onion epidermis, which was killed with alcohol and washed with water; and then were fixed and stained in the same manner. This method was suggested by Dr. KUSANO of the Tokyo Imperial University. In using the paraffin method, when the material was cleared with xylol the filaments were put together as straight as possible, and then were kept under a light slide in a petri dish. During infiltration with melted paraffin, the material was kept also under a heavy cover glass in the dish.

Most of the material was stained with Haidenhain's iron-alum haematoxylin, but some with Magdala red and anilin blue; and mounted in Venetian turpentine. Paraffin sections were cut 5 or 6μ thick and stained with Haidenhain's iron-alum haematoxylin.

CELL DIVISION

For this purpose, *O. grande*, collected from Palos Park, was used. Throughout this account the correlation between the somatic mitosis and the development of the ring will be traced, but first a brief sketch is given of the resting nucleus of the vegetative cell to contrast with the various changes during the division.

RESTING NUCLEUS.—The vegetative cells are cylindrical, containing peripheral reticulate chromatophores in which many pyrenoids and minute starch grains are scattered. The very small starch grains have been described as the first visible product of photosynthesis. Each pyrenoid is also surrounded by somewhat larger starch grains derived directly from the pyrenoid. The resting nucleus is generally circular and flattened in shape, and lies in the peripheral protoplasm a little more deeply placed than the chromatophore, and at the same distance from both cross walls of the cell (fig. 1). The nucleus has a distinct nuclear membrane, within which is a very fine reticulum. At the nodes of the reticulum there is an abundance of chromatin; and there are one or more nucleoli (fig. 2).

MIGRATION OF NUCLEUS AND APPEARANCE OF RING.—The first indication of cell division can be observed in the migration of the nucleus toward the upper end of the cell, until it reaches a point about two-thirds of the distance from the base to the top of the cell (fig. 3). Then the nucleus begins to enlarge, usually changing its shape from circular to ellipsoidal or spindle-shaped, but without any particular change in its structure. While the elongated nucleus is still in the same condition, a very narrow ring appears along the longitudinal cell wall at the extreme upper end of the cell. First it looks something like a streak connected with a tiny elevation originating from the second layer of the cell wall (fig. 4); but as it develops one can easily find a fine circular splitting on the surface of the ring. The free edges of the ring at the splitting are in connection with the second layer where the elevation has appeared.

PROPHASE.—The first step of the prophase begins by the breaking down of the reticulum, showing some lighter areas at certain regions; and is followed by the appearance of the very irregular zigzag threads of chromatin and reticulum of uneven thickness everywhere in the nucleus (fig. 5). Uniting with each other, denser masses of chromatin begin to condense into the chromatic threads of even thickness, while the lighter spaces become wider and clearer by the disappearance of the reticulum (figs. 6, 7). Gradually these threads begin to straighten, and finally form a single slender and coiled chromosome thread, the so-called spireme. At this stage the nuclear membrane has disappeared.

The longitudinal splitting of the chromosomes appears in these slender threads, beginning as small vacuoles along the axis, but later developing a more or less continuous split resulting in two parallel strands (fig. 8). These strands become shorter and thicker and finally are segmented into a definite number of chromosomes. At this stage the longitudinal split becomes obscured by the close association of the halves, which, however, still can be seen (fig. 9). While these chromosomes are at their highest contraction the spindle is formed in the cytoplasm, consisting of fine fibers and numerous minute granules.

RING IN PROPHASE.—During the nuclear prophase the ring grows rather rapidly, increasing its diameter to five or six times that of the

earliest stage; and also its wall becomes thicker, showing obscurely two layers. The split of the ring increases its width, and it becomes clearly visible at the point of attachment to the second layer of the cell wall.

METAPHASE.—The chromosomes now lie close together at the equator of the spindle, forming the so-called equatorial plate. At this stage they show a great diversity in their forms and sizes. The forms may be classified into three main groups: short rod, long rod, and loop; while the sizes are entirely different. It is not difficult to trace the longitudinal splitting along the axis in some chromosomes (fig. 10). The spindle attachments of the chromosomes are correlated with their various differences in shape. These different attachments may be grouped into terminal, subterminal, median, and submedian, according to WILSON'S classification (fig. 11).

ANAPHASE.—Each double chromosome begins to separate at the place where the fibers are attached, and the two parts gradually move from the equatorial plate (fig. 12). Then both groups become entirely free and proceed to opposite poles of the spindle, showing the characteristic forms of the individual chromosomes (figs. 13, 14). As soon as these daughter chromosomes reach the poles they begin to contract into dense masses, in which the individual chromosomes can still be distinguished in some degree (fig. 15).

RING IN METAPHASE AND ANAPHASE.—Although it is difficult to name any particular stages in growth during these periods, the ring continues its growth in the same way as in the preceding period; that is, at the end of the anaphase it increases its diameter to about seven or eight times that of the first appearance, and there is a thickening in both layers of its wall. The portions of the free edges of the ring where they are in contact with the cell wall become so evident that one can observe them without difficulty. The ring seems to have some kind of mucilage in its cavity which stains dark with iron-alum haematoxylin.

TELOPHASE.—After remaining some time closely crowded together at the extreme ends of the spindle, the chromosomes of each daughter nucleus begin to separate from one another to reconstruct the resting nucleus. At the same time their boundaries become scarcely visible, and within each chromosome minute vacuoles arise

along its axis (fig. 16). While these vacuoles are developing the volume of the nucleus increases, but it is still smaller than the ordinary resting nucleus.

Both daughter nuclei now approach each other at the equator of the spindle (fig. 17), and when they come almost into contact, numerous fine granules appear around them. These fine granules gather together, forming a thin wall between the nuclei. At this stage in the nucleus a more and more uniform reticulum is found, in which the nucleolus begins to appear, while the limits of the disappearing chromosomes still can be recognized (fig. 18).

The nuclear membrane seems to make its appearance during this stage. After formation of the separating wall the daughter nuclei move apart and take their position at the center of the peripheral protoplasm. The nucleus shows all structures of the resting condition (fig. 19).

RING IN TELOPHASE.—At the end of this stage the ring probably reaches its full growth, increasing 1–2μ in diameter, and there is also some increase in thickness. The opening of the split in the ring becomes wider and very conspicuous.

DEVELOPMENT OF RING INTO NEW CELL WALL.—After remaining for a while in the same position the upper nucleus moves near the ring, which then begins to stretch. Between the stretching ring and the outer layer, the mucilaginous mass is sometimes observed (fig. 20). The portion of the outer layer which was associated with the stretching ring begins to break up, leaving zigzag lines at both edges (fig. 21). The stretching is followed by a straightening, probably caused by the turgidity of the cell contents.

At the same time the separation wall between the new cells moves up and unites with the inner layer of the cell wall at the joining of the new and the old wall (fig. 22). The layers shown in the wall of the ring now become the outer and inner layers of the new cell wall, although they are still very thin. This thin new cell wall continues its growth until it reaches the usual length and thickness.

SPERMATOGENESIS

Antheridia are borne in two kinds of filaments. In macrandrous species they are borne in the same filament with the oogonia, or in

dioecious species in filaments of about the same size as the female filaments. In nannandrous species the male filaments are so small that they are called dwarf males, and they are epiphytic on the female filaments, usually on the suffultory cell. The formation of antheridia is so different in the two types that they will be described separately.

MACRANDROUS TYPE

In this type there are two methods of forming antheridia. In one, *O. grande*, the antheridia are formed in a long series which may consist of more than thirty antheridial cells occurring at one or more places in a filament. In the other there is a short series consisting of a few cells arising successively. The essential features in the formation of antheridia in the two cases are sufficiently similar to be described together, however, except in the mode of formation of sperms.

FORMATION OF ANTHERIDIA.—At the beginning of the formation, the antheridia of *O. grande* show the same migration of the nucleus toward the upper end of the cell which was observed in vegetative cell division. In this case, however, the nucleus almost reaches the cross wall. Then a small ring makes its appearance at the same place; but it does not grow as much as in vegetative cell division. The mitosis now takes place just as in somatic division (fig. 23). As a consequence of this division there is formed a small antheridial cell at the upper part. This process is repeated by the larger antheridial mother cell (fig. 24).

CELL DIVISION IN ANTHERIDIA.—After the antheridial cells reach a certain number, each of them begins to divide into the two sperm mother cells. During this division all important phases of the mitosis can be seen without difficulty (figs. 23–26). The most striking feature in this is that the figure is not oriented along the vertical but along the horizontal axis of the cell, probably because the cell is so short in proportion to its length. The result of this arrangement is that at first the two daughter nuclei lie side by side (fig. 25*d*), although the cell is to be divided transversely; so the stretching of the ring begins before the formation of the separation wall (figs. 25, 26). This feature is also different from the somatic mitosis. As soon as the ring has stretched, one of the daughter nuclei moves upward,

where there is more room (figs. 25, 26), and then a thin wall begins to appear between the nuclei (fig. 26*w*). In this way the sperm mother cell is formed.

FORMATION OF SPERMS.—The sperm mother cells start to form the two sperms in each cell, lying side by side. So far as the mitosis is concerned, this cell division follows the same method observed in the antheridial cell, although it is too small to show details (figs. 27, 28). But the distinguishing differences are that there is no formation of a ring, and also that the sperms are developed from the two daughter cells. After the formation of cilia is completed the swimming sperms escape from the cells (fig. 29).

NANNANDROUS (DWARF MALE) TYPE

ANDROSPORANGIUM.—Preceding the formation of the dwarf male, the androsporangium appears in the female filament at a short distance above the oogonium in *O. nebraskense* (12). It is formed by a typical vegetative *Oedogonium* cell division, but is much smaller than the vegetative cell. There may be a single androsporangium, or two, or sometimes three or four in a series. When the androspores are mature they swim about for a while, and then attach themselves to the suffultory cell where they develop into the dwarf males (fig. 30).

DWARF MALE.—The androspore forms first a basal vegetative cell, the so-called stipe (fig. 31), which gives rise to a ring at the top of the cell (fig. 32). The nucleus then divides as usual (fig. 33), and by stretching of the ring the first antheridial cell is formed at the top of the stipe with a cap at its head (figs. 34, 35). After the nucleus in the antheridial cell has remained in the resting stage for a time (fig. 36) it begins to form the two sperms, separated transversely by a thin cross wall (figs. 37, 38). Then a second ring arises in the stipe near the place of formation of the first one (fig. 39). Sometimes it appears before the first antheridium has given rise to the sperms. After nuclear division the second antheridial cell is formed just beneath the first one (fig. 40). The nucleus in the second antheridial cell divides into two and another pair of sperms is discharged (fig. 41).

At the beginning of the formation of the sperms usually the new

daughter cells begin to contract in the antheridial cell. The nucleus takes a position at some corner of the cell, leaving a clear space at the opposite side, in which something like the blepharoplast appears first as a circle of granules just under the plasma membrane. Each granule then seems to give rise to a cilium. The antheridial cell of the dwarf male being much longer than that of the macrandrous type, development of the cilia can be recognized with comparatively less difficulty (figs. 39–43). While the second antheridium is still forming the sperms the first is generally discharging them.

Oogenesis and fertilization

Oogenesis

The oogonium may develop from any of the vegetative cells, but most frequently it arises from a cell well supplied with chlorophyll, starch grains, and other food substances, and is called the mother cell. There are two types of mother cells; the one having characteristic swellings of its longitudinal cell wall (as in *O. nebraskense*) is easy to recognize (fig. 44), while in the other type (*O. americanum*), apparently without any such modification, it is rather hard to distinguish whether it is going to be a mother cell or an ordinary vegetative one. If more attention is paid to the cells, however, there may be found some differences between them.

In the first place, the mother cell is usually longer than the ordinary vegetative cell; and also, when the vegetative cell divides, the nucleus generally migrates toward the upper extremity of the cell; and as soon as the ring begins to form the nucleus passes into the mitotic process. But in the mother cell, even after the formation of the ring, the nucleus not only remains in the resting stage for a rather long time but it stays at the center of the cell (fig. 44). Besides these features the contents of the mother cell in general are much denser than those of the vegetative cell.

This nuclear situation is very evident in *O. nebraskense*. In this species, when the ring attains its full growth the nucleus moves slightly in the opposite direction (fig. 45). The reasons the behavior is different from that in the vegetative cell division may be partly physiological, because the nucleus has to keep its activity longer for manufacturing food substances for the young egg; and partly mor-

phological, because the nucleus has to stay where the cell division takes place.

In oogenesis the ring naturally becomes more conspicuous than in spermatogenesis, because the oogonium is larger than the antheridium (fig. 46). While the cross wall is being formed between the two daughter nuclei the ring begins to stretch, and as soon as the wall of the oogonium is formed by the rupture of the ring, the greater part of the contents of the mother cell moves into the oogonium, accompanying the cross wall to the joining point of the new and old cell walls, and leaving large vacuoles behind in the old cell, which is now called the suffultory cell (figs. 47–52). When the oogonia are formed, the second one is always produced by the suffultory cell, forming the second ring just beneath the first oogonium (figs. 53, 54). After attaining a certain size, a pore or split is formed at some part of the oogonium wall; and the contents of the oogonium begin to constrict and round off, forming a single oosphere (fig. 52).

It is difficult to observe the nuclear behavior of the mitotic process, since the contents of the mother cell are so dense, but probably it acts just as in the vegetative cell division.

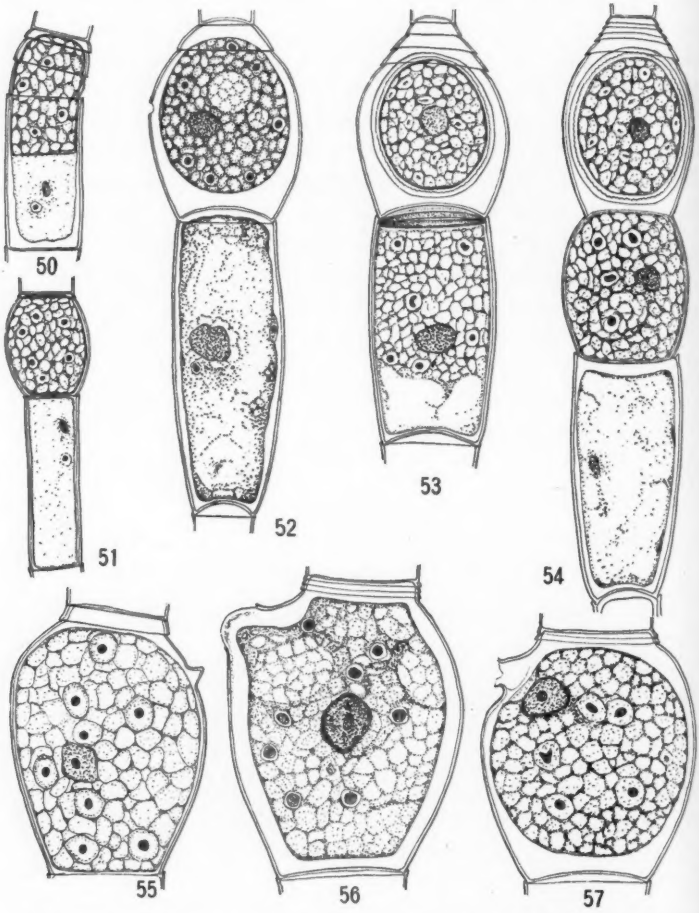
Fertilization

Fertilization has been so well described by previous investigators, especially Klebahn (**8**), that an account of it need not be repeated here; but brief mention is made of what has been observed in *O. americanum*.

When the oosphere is ready for fertilization, it forms a pore at a certain point on the oogonium wall (figs. 55–57). The nucleus of the oosphere, which is in the resting stage, approaches its surface, facing the opening in the oogonium wall (fig. 57), which the swimming sperm then enters. In some species there seems to be nothing to hinder the sperm from entering the oogonium, because of the large opening (fig. 58). Then the sperm penetrates the oosphere by pushing the cell contents inward (figs. 58, 59).

Fusion of the two nuclei now takes place. The male nucleus is always smaller than the female and has only chromatin granules in its reticulum; while the female nucleus has in addition a distinct nucleolus. With both nuclei in the resting stage, a fusion of all the

nuclear material takes place (figs. 59, 60). As the process of fusion advances, both nuclear membranes, which have been in contact with each other, show in their final stages some irregular remains, out-



FIGS. 50–57.—Figs. 50, 51, various stages of oogenesis without suffultory cell; fig. 52, formation of pore and oosphere; fig. 53, second ring in suffultory cell; fig. 54, formation of second oogonium; figs. 55, 56, pore at upper part of oogonium cell wall; fig. 57, migration of nucleus toward pore.

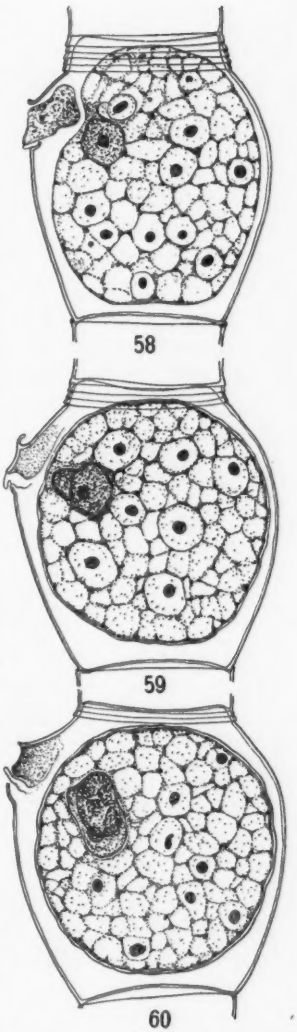
lining the form of the fusing nuclei (figs. 60, 61), which then begin to return to the center of the fertilized egg. When fusion is completed the nucleus generally shows a circular outline.

FORMATION OF SPORE COAT.—After penetration of the sperm into the egg the spore coat begins to develop. The first indication of its formation is the appearance of the numerous granules around the egg under the thin outer layer (fig. 61). Next the second wavy layer is formed of a certain thickness, the wide, smooth, inner part being added by deposition from the contents of the egg (figs. 62, 63). Finally the third or inner layer, which is rather smooth and thin, also arises from the granules deposited by the fertilized egg (fig. 64).

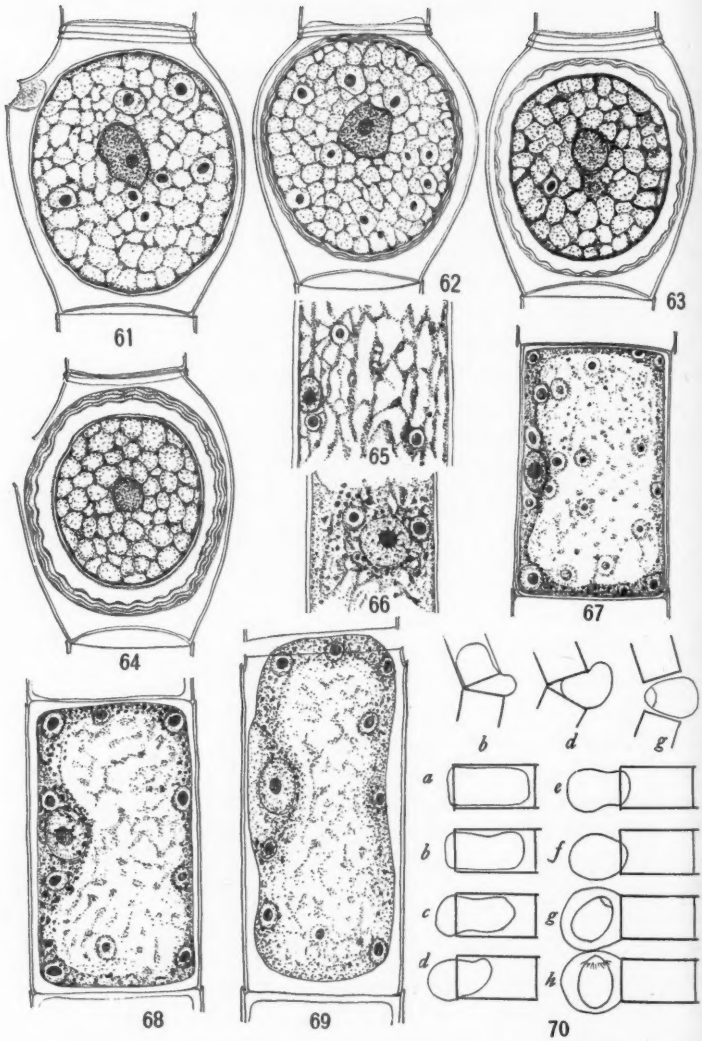
During formation of the middle and inner layers the outer layer increases in thickness. It is interesting to compare the thickness of the cell wall and the diameter of the fertilized egg, for the more the thickness of the cell wall increases the more the diameter of the egg decreases.

ZOOSPORE

Asexual reproduction takes place by means of zoospores which arise singly from any of the ordinary vegetative cells. The cell which is to produce a zoospore contains such abundant contents that the cavities between the reticulate chromatophores



FIGS. 58–60.—Fig. 58, entrance of sperm into oogonium; figs. 59, 60 fusion of male and female nuclei in resting stage.



FIGS. 61–70.—Fig. 61, fusing nuclei returning to center of egg, and appearance of numerous granules around egg for formation of spore coat; fig. 62, formation of wavy part of second layer of spore coat under thin first layer; fig. 63, formation of smooth part of second layer; fig. 64, formation of third inner smooth layer; fig. 65, position of nucleus in vegetative cell; fig. 66, sinking of nucleus beneath clear membrane; fig. 67, appearance of granules of different sizes around nucleus; fig. 68, nucleus deeply placed under clear space, with granules gathering along margin; fig. 69, splitting of cell wall for escape of zoospore; fig. 70, topographic figures showing escape of zoospore from cell.

begin to disappear, and the peripheral protoplasm becomes much thicker, decreasing the size of the large central vacuole.

The first difference recognized in the nucleus is a change in its position. In the ordinary vegetative cell it is in the peripheral protoplasm a little more deeply placed than the chromatophore (fig. 65); but now it moves inward, leaving a circular light area beneath the clear membrane (fig. 66). During this process the nucleus increases in size, but does not show any particular change in its constitution. Granules of different sizes begin to appear around the nucleus and gradually increase in number (fig. 67). Finally the fine granules gather much more densely along the margin of the circle (fig. 68); and it seems probable that the blepharoplast may arise from these granules, although this is still to be proved.

ESCAPE OF ZOOSPORE.—It is interesting to watch the zoospore escaping from the cell. Just before it does so there appears an active Brownian movement of some small particles in the protoplasm at the upper end of the cell, where the splitting of the cell wall is going to take place (fig. 69). As soon as the cell wall splits transversely, one end of the mass of cell contents begins to emerge from the slit, and at the same time the other end begins to round off, separating from the cross wall of the cell (fig. 70*b*). Gradually the whole mass escapes from the cell and shows an amoeboid movement (fig. 70*c-e*). When the whole body has emerged it begins to round off, and assumes the characteristic pyriform shape of the zoospore (fig. 70*d-f*), which at first is surrounded by a delicate hyaline bladder. While it is still in the bladder the numerous granular cilia become visible in a circle, at the colorless narrower end (fig. 70*g*), and then suddenly the cilia are spread out in a radiating arrangement (fig. 70*g*, *h*). The position of the cilia is always at a right angle to the longitudinal axis of the old cell. Then the zoospore begins to swim quickly. The entire process lasts only a few minutes, but the swimming stage lasts probably about one or two hours. This process was observed in the unknown species.

HABIT OF ZOOSPORE.—There may be several factors to stimulate formation of zoospores, but a change in temperature seems to be most effective. Whenever material was brought either from a warm to a colder place, or from a cold to a warmer place, numerous zoo-

spores appeared in two or three days. In August, for instance, after keeping material in the refrigerator for three days it was put on the window sill. Then the zoospores began to swim from early morning until late afternoon for about two days. Some material, which had been kept in Knop's solution for some time, was put into distilled water for two days and the same experiment repeated. At that time the formation of zoospores was so abundant that almost all filaments were broken into small pieces, after three days, by producing the numerous zoospores. Another example was noted on November 25, 1924. It was so cold that all jars of *Oedogonium* placed on the outside of the window began to freeze; so they were taken into the warm room. After five days each jar showed a remarkable number of zoospores. Whenever the material was put in Knop's solution or taken from it to distilled water, some zoospores were formed within two or three days. The same thing happened when the material collected from the field was washed with water to take off the dirt. While the zoospores are swimming they show heliotropism distinctly, and rest themselves at the surface of the water along the jar.

Discussion

The first cytological investigation of cell division in *Oedogonium* was by STRASBURGER (**19**), working with *O. tumidulum* Kg. in 1880. He described the origin of the ring, the whole process of nuclear division, and the formation of the cell wall. In the nuclear division he described some phases, but did not include the behavior of the chromosomes, a feature which was studied more accurately in 1908 by WISSELINGH (**23**), who also counted the chromosome number in *O. cyathigerum*. KLEBAHN (**8**) investigated the nuclear division in *O. boscil*. These investigators, comparing the mitosis of *Oedogonium* with that of higher plants, like *Tradescantia* or *Lilium*, agreed in finding striking resemblances between them.

It is not necessary to repeat the same details here, since they can only be confirmed, but there may be some small differences in my material. The one thing which should be mentioned is that my material, *O. grande*, shows more details in the mitotic figures. In the first place, modern methods of technique show structures more accurately than the former ones; and also there are surely differences

in the different species. Treating the different species with the same methods produces quite different results: some species show distinct figures; others show them obscurely; and some do not show anything, probably on account of abundant cell contents.

WISSELINGH (**23**) counted nineteen chromosomes in *O. cyathigerum;* I found thirteen in *O. grande*. He considered it a matter of interest that there was an uneven number in the species which he studied, a feature which he supposed to be very rare in the plant kingdom. I also found the forms of chromosomes in a figure to be as various as he described them in his species.

In regard to the origin, structure, and growth of the ring, as well as the formation and behavior of the separating wall between the two daughter cells, there have been various theories discussed by almost all the workers who have investigated *Oedogonium*, especially PRINGSHEIM (**16**), STRASBURGER (**19**), KLEBAHN (**8**), WILLE (**21**), HIRN (**6**), and WISSELINGH (**24**). The history of these investigations is well described by WISSELINGH, who also worked out the features in detail. Nothing entirely new was found in *O. grande*, but the investigation confirms especially WISSELINGH's descriptions of the origin of the ring, its manner of stretching, and the formation and behavior of the separating wall between two daughter cells.

Spermatogenesis was studied chiefly by PRINGSHEIM (**16**), JURANYI (**7**), KLEBAHN (**8**), and HIRN (**6**), but the descriptions were mostly morphological, not cytological. KLEBAHN described the details of formation of the antheridial cell, however, and made some remarks on the mitosis, but not in detail. Some figures of *O. grande* resemble his illustration, but differ in having the formation of a ring, and also in not having both vertical and horizontal divisions in the antheridial cells of the same filament. According to his explanation, an antheridium is forming sperms in both ways, namely, by vertical and horizontal divisions; while in my material another antheridial cell is being produced. In *O. grande* each primary antheridial cell produced by the mother cell usually forms another secondary antheridial cell, the so-called sperm mother cell with the lateral wall developed by a ring. This antheridial cell forms two sperms by a vertical division without forming a ring. My material fortunately shows an almost complete series of the mitosis in both the formation of

antheridia and sperms; although in the latter the figures are not so distinct as in the former, owing to the narrowness of the cells.

The most striking features are exhibited by the mitotic figures in the division of the antheridial cell. In this case the ring is formed as usual, but the mitotic figures lie along the transverse axis, in spite of the fact that horizontal cell division is going to take place. The reason may be that the length of the cell is too short to let the figures lie longitudinally. But finally, when the ring stretches out, one of the nuclei migrates into the new space. It seems to be an interesting phenomenon from the point of view of adaptation. Even minute figures like these adapt themselves to the environment for some time in the process of division.

In the formation of sperms, although the same mitotic process is repeated, one thing is different from typical *Oedogonium* cell division: there is no formation of a ring. The reason seems to be that the cell itself has already been formed, consequently division takes place only in the cell contents.

PRINGSHEIM, HIRN, and others investigated the development of dwarf males, but they did not try to trace the whole process step by step. The dwarf male is so small that it is hard to study the mitosis in detail, but a morphological tracing can be made without difficulty. The dwarf males of *O. nebraskense* form in general two antheridial cells; the upper one is always older than the lower, and each produces two sperms without the formation of a ring.

It is of interest to compare this nannandrous type with the macrandrous type in their methods of spermatogenesis. In the nannandrous type, the stipe corresponding to the antheridial mother cell produces only antheridial cells, which in this case never form another secondary antheridial cell. In the macrandrous type, however, both the antheridial mother cell and antheridial cell are generally able to form another antheridial cell. The length of the antheridial cell of both types is quite different, being much longer in the nannandrous type; therefore this might be the best form to study for the origin and development of the cilia of sperms. There is more space and comparatively less cell contents.

In oogenesis the principal work has been done by PRINGSHEIM, KLEBAHN, and HIRN, but in most of the forms studied there was no

suffultory cell; consequently I have described oogenesis with a suffultory cell to compare with the other accounts. The behavior of the nucleus is interesting, because it does not migrate at all toward the upper end of the cell, but nevertheless shows how active it is as the center of oogenesis.

Fertilization was described by PRINGSHEIM, JURANYI, KLEBAHN, and HIRN. KLEBAHN furnished an excellent description from the cytological point of view, which is confirmed by my observations in *O. americanum*. I have added a brief sketch of the formation of the cell wall of the fertilized egg, using the same species, which is quite different from other cases of cell wall formation in *Oedogonium*.

Formation of the zoospore has also been worked out by various investigators, among them STRASBURGER (**18**), who studied its cytology. He mentioned repeatedly the change of position of the nucleus during the various stages of development of the zoospore. My material, probably owing to the different species, shows scarcely any such changes of the nuclear position, except a slight migration inward from the peripheral position, where a clear circle appears. The larger the clear circle becomes, however, the deeper the nucleus sinks. The nucleus always accompanies the clear circle, never moving apart from it; consequently no radial substance was found between the nucleus and the circle, as STRASBURGER observed; but numerous coarse and fine granules were found around the circle, as if they were going to form a blepharoplast, as DAVIS (**3**) suggested in his study of *Derbesia*.

Germination of the fertilized egg was searched for constantly, but was not found in material just brought in from the field, and could not be induced by any of the methods which have proved successful in forcing the germination of seeds. No doubt reduction of chromosomes takes place at the germination of the egg. No one has ever observed the nuclear conditions at the germination of the egg of *Oedogonium*, or of the nearly related *Bulbochaete*.

Summary

1. Although the somatic mitosis of *Oedogonium* has already been shown by STRASBURGER and WISSELINGH, *O. grande* shows the figures more distinctly than their illustrations, furnishing a complete

series of the mitotic process, especially the behavior of chromosomes which show a remarkable resemblance to those of higher plants. This is due partly to modern methods of technique and partly to the species studied.

2. The number of chromosomes of this species is thirteen, an uneven number, in this respect agreeing with the uneven number nineteen in *O. cyathigerum* counted by WISSELINGH.

3. In the spermatogenesis all important phases of the mitosis in the formation of both antheridia and sperms have been traced in *O. grande*. The most striking feature is found in the mitosis of the antheridial cell, in which the figure is oriented along the horizontal axis, while the cell division is to be transverse. But when the ring stretches, one of the daughter nuclei, which have been lying side by side, moves into the upper space and becomes separated by a cross wall. This seems to be an unusual condition in cell division and has never been described in *Oedogonium*.

4. The complete series of spermatogenesis of the dwarf male was found in *O. nebraskense*, which shows the formation of sperms very distinctly.

5. There are two types of oogenesis, one with a suffultory cell (*O. nebraskense*) and the other without (*O. americanum*). The behavior of the nucleus in oogenesis is different from that in the vegetative cell division, in staying in the same position or in migrating slightly toward the lower end of the cell, while the nucleus in the vegetative cell division migrates toward the upper end of the cell. Both show that they are the center of morphological and physiological activity.

6. As soon as fertilization takes place in *O. americanum* the spore coat develops from the fertilized egg. The spore coat consists of three layers: a thin and smooth outer one; a thick, half wavy and half smooth middle one; and a thin and smooth inner one.

7. In the formation of the zoospore, the behavior of the nucleus of the unknown species is a little different from that of *O. tumidulum* described by STRASBURGER, in not changing its position according to the various stages of the development of the zoospore. The nucleus of my material sinks just under the clear circle in which the cilia of the zoospore appear. The process of escape of the zoospore from the

cell takes only a few minutes. Of the factors which stimulate the formation of the zoospore, change of temperature seems to be most effective so far as the present experiments are concerned.

Professor CHARLES J. CHAMBERLAIN suggested that I undertake this work, and I wish to express to him my gratitude for his valuable suggestions and kindly encouragement. I am also indebted to Professor E. N. TRANSEAU, who examined the material and determined the species.

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EXPLANATION OF PLATES I–III

FIGS. 1–28 (except fig. 3), ×790; figs. 3, 29, ×380; figs. 31–43, ×800; figs. 30, 44, 46, 47, 49, 52, ×390; figs. 45, 48, 50, 51, 53, 54, ×350; figs. 55–69, ×800.

FIGS. 1–28 (except figs. 20, 21, 23), *O. grande;* figs. 20, 21, 23, 29, unknown species; figs. 30–54 (except figs. 50, 51), *O. nebraskense;* figs. 50, 51, 55–64, *O. americanum;* figs. 65–70, unidentified species.

FIG. 1.—Vegetative cell with reticulate chromatophore.

FIG. 2.—Resting nucleus.

FIG. 3.—Migration of nucleus toward upper end of cell.

FIG. 4.—Elongation of nucleus and appearance of young ring.

FIG. 5.—Prophase: irregular zigzag threads of chromatin and reticulum of uneven thickness.

FIGS. 6, 7.—Condensation of chromatin threads to even thickness.

FIG. 8.—Single slender and coiled spireme splitting longitudinally by small vacuoles.

FIG. 9.—Segmentation of chromosomes and appearance of spindle.

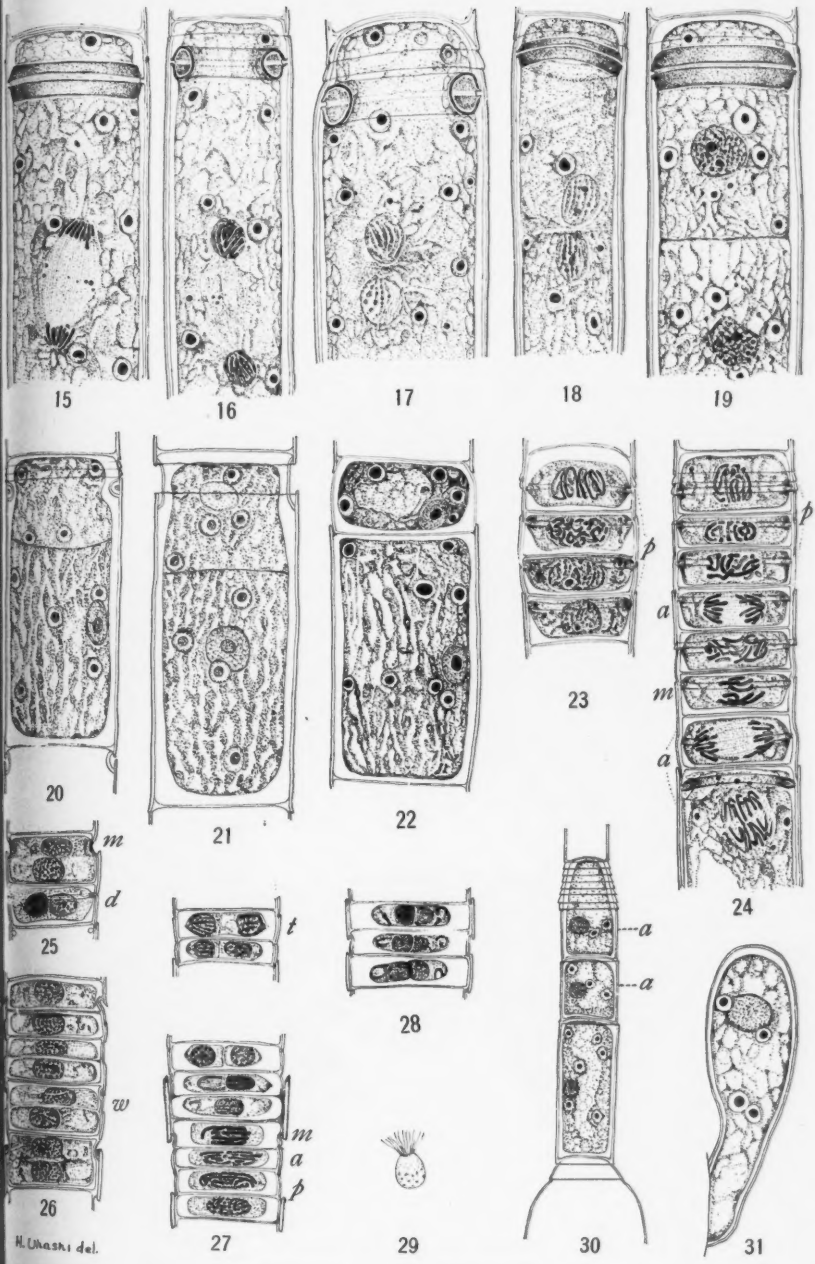
FIG. 10.—Metaphase: gathering of chromosomes at equator of spindle.

FIG. 11.—Various forms of spindle attachment.

FIG. 12.—Anaphase: separation of double chromosomes.

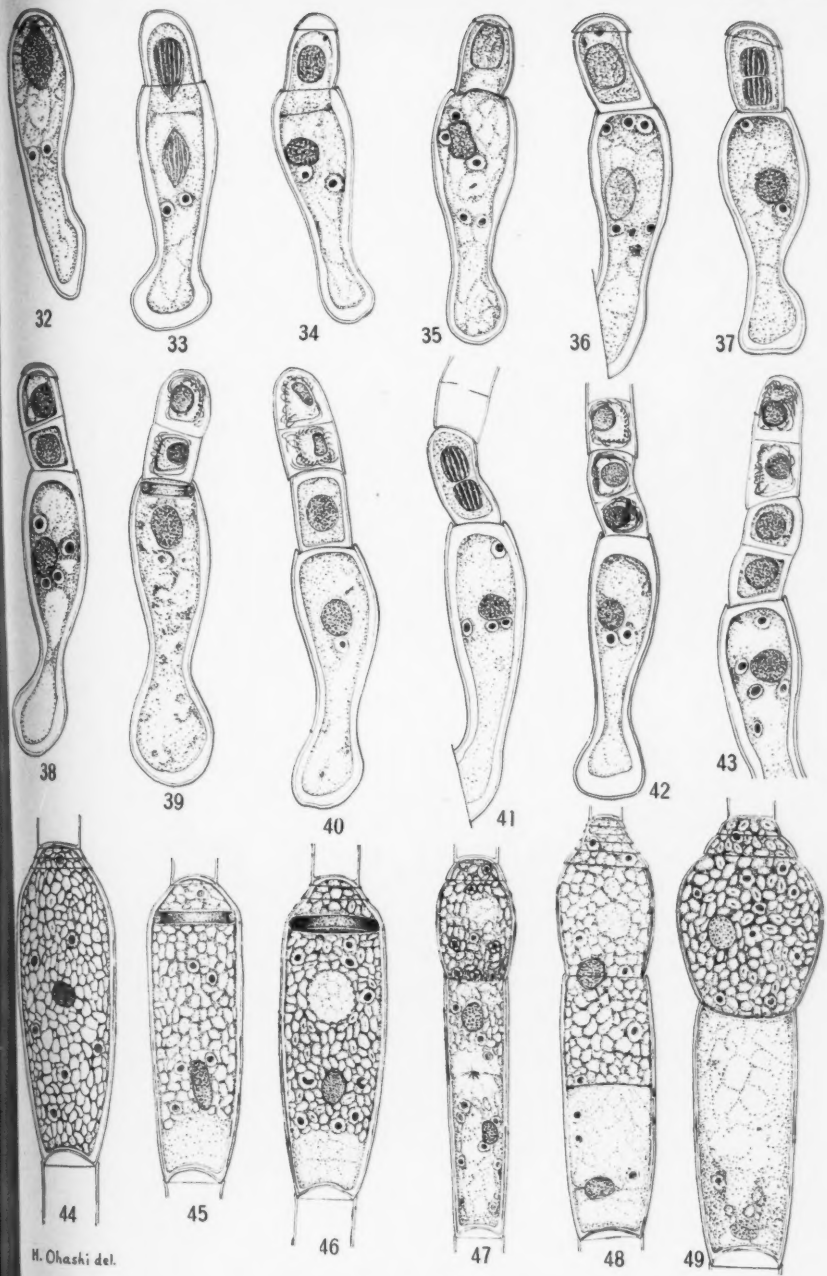
FIGS. 13, 14.—Migration of chromosomes to poles of spindle.

OHASHI on OEDOGONIUM



OHASHI on OEDOGONIUM

BOTAN



OHASHI on OEDOGONIUM

FIG. 15.—Contraction of chromosomes into masses.

FIG. 16.—Telophase: separation of chromosomes by vacuoles along axis for reconstruction of resting nucleus.

FIG. 17.—Approach of two daughter nuclei at equator of spindle.

FIG. 18.—Appearance of nucleolus in more or less uniform reticulum and formation of cross wall between two nuclei.

FIG. 19.—Separation of nuclei after formation of cross wall.

FIG. 20.—Beginning of stretching of ring under outer layer of cell wall, showing some mucilage at outside.

FIG. 21.—Broken outer layer of cell wall.

FIG. 22.—Straightening of ring and migration of cross wall upward to unite with inner layer of cell wall.

FIGS. 23, 24.—Mitotic figures lying horizontally in antheridial cells with formation of ring; showing various stages in prophase (*p*), metaphase (*m*), and anaphase (*a*).

FIG. 25.—*d*, Two daughter nuclei lying side by side in antheridial cell; *m*, migration of one nucleus upward after stretching of ring.

FIG. 26.—Formation of cross wall between two nuclei lying one above the other.

FIG. 27.—Mitosis in sperm mother cell showing prophase (*p*), metaphase (*m*), anaphase (*a*), and telophase (*t*).

FIG. 28.—Formation of sperms.

FIG. 29.—Swimming sperm.

FIG. 30.—Androsporangia (*a*) of *O. nebraskense*.

FIGS. 31–36.—Dwarf males of *O. nebraskense* in various stages of formation of first antheridial cell.

FIGS. 37, 38.—Formation of sperms in first antheridial cell.

FIG. 39.—Formation of second ring in stipe, and ciliated sperms in first antheridial cell.

FIG. 40.—Formation of second antheridial cell.

FIG. 41.—Formation of sperm in second antheridial cell.

FIGS. 42, 43.—Formation of sperms.

FIG. 44.—Oogonium mother cell of *O. nebraskense* with nucleus at center.

FIG. 45.—Formation of ring at top of oogonium mother cell and migration of nucleus toward lower end of cell.

FIG. 46.—Ring at full growth in mother cell.

FIGS. 47–49.—Various stages of oogenesis with suffultory cell.

SPECIALIZATION IN SECONDARY XYLEM OF DICOTYLEDONS

II. EVOLUTION OF END WALL OF VESSEL SEGMENT

FREDERICK H. FROST[1]

(WITH SIXTEEN FIGURES)

Introduction

The origin of the vessel was discussed in the initial paper of this series (4). It was found that the vessel segment of the arboreal dicotyledons developed phylogenetically from a typical scalariform tracheid by the loss of pit membranes at the ends of the tracheid. The specialization also resulted in the formation of clearly defined end walls. The present paper continues the discussion from this point, and traces the further specialization of the primitive scalariform perforation. The first paper includes an explanation of the methods which were used in this and the following studies.

BOODLE and WORSDELL (2) described transitions from scalariform to simple perforations which they had observed in the secondary xylem of several dicotyledons. Although they did not state that the sequence reflects the phylogenetic origin of the porous vessel segment, this may be inferred as their viewpoint. JEFFREY (5) described an identical transition from *Vaccinium*. He concluded, from a consideration of the sequence, that the simple perforation originates from the scalariform perforation by the gradual loss of the bars of the latter. Later THOMPSON (6), studying *Vaccinium*, corroborated the interpretation of JEFFREY, and agreed that the sequence is palingenetic and that it illustrates the origin of the porous vessel segment.

BROWN (3) described similar transitions which he had observed in positional sequence from the protoxylem to the metaxylem. BAILEY and TUPPER (1) showed conclusively that the scalariform perforation is primitive and that the simple perforation is specialized. This proved, for the first time, that the sequence is truly palingenetic

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and that the direction of the specialization is from the scalariform to the simple condition.

Despite contradictory opinions as to the origin of the vessel, there is uniformity of opinion concerning the further specialization of the end wall. In the first place, the wide occurrence and uniformity of the sequence in many different plants indicate its palingenetic character, and, in the second place, there is only one rational manner in which to interpret the direction of the sequence. The present paper presents additional evidence in favor of this well established view, and outlines the details of this line of specialization.

Discussion

Dicotyledonous woods may be classified into four major groups on the basis of the type of vessel perforation. These are: (1) woods with vessels which have exclusively scalariform perforations; (2) woods with both scalariform and simple perforations in different vessel segments; (3) woods with oblique simple perforations and with vestiges of the scalariform condition in the smaller vessel segments; and (4) woods with vessels which have transverse porous division walls. The scalariform-porous woods may be dominantly scalariform, as is typical of *Myrica inodora*, or dominantly porous, a condition found in *Sambucus simpsonii* and many other species.

To obtain the evolutionary sequence of these four types, it is necessary to correlate them with a characteristic whose evolutionary history has been established. The writer showed, in the first paper of this series (4), that specialization resulted in a decided decrease in the length of the vessel segment. Using length of vessel segment as a base, the results given in table I were computed from data taken from BAILEY and TUPPER (1).

Table I shows that with increasing specialization of the vessel segment, the perforations of the end walls change from the scalariform to the simple type. It may be concluded, therefore, that the scalariform perforation is primitive and the porous perforation specialized, and that the phylogenetic order of development is from scalariform, to scalariform-porous, to oblique-porous with vestiges of the scalariform condition, to transverse porous. Scalariform-porous species with dominant scalariform perforations are, in regard

to this particular characteristic, more primitive than those with dominant porous perforations.

If the assumption is made that the vessel segment is a modification of the tracheid, it follows, by the method of association, that primitive vessel segments will possess the general features of tracheids. It also follows, by the method of correlation, that characteristics which correlate with these tracheid-like features will themselves be primitive in the organization of the vessel segment. The

TABLE I

AVERAGE LENGTH OF VESSEL SEGMENTS

TYPE OF PERFORATION	NO. OF SPECIES	LENGTH (MM.)
I. Entirely scalariform	52	1.09
II. Scalariform porous	19	0.81
III. Oblique porous	34	0.69
IV. Transverse porous	169	0.41

TABLE II

CORRELATIONS OF PRIMITIVE CHARACTERISTICS WITH TYPE OF PERFORATIONS

TYPE OF PERFORATION	NO. OF SPECIES	DIAMETER (MM.)	PERCENTAGE		
			Outline angular	Thin walls	Evenly thickened walls
I. Scalariform	40	0.067	100	100	97
II. Porous (transverse)	40	0.120	15	25	22

general features of tracheids are, in part, small cross-sectional diameter, angularity of outline, thin walls, and evenly thickened wall. The measurements summarized in table II were made as a check on the length-perforation correlation.

In every case the scalariform condition correlates with the primitive characteristic while the specialized or porous condition of the end wall shows little correlation. In addition to this evidence, many species show the transition from the scalariform to the simple perforation when a traverse is made from the protoxylem to the secondary xylem. This transition is also found in the secondary xylem, and is illustrated in fig. 1 from *Myrica inodora*.

There are five major factors which should be considered in a detailed investigation of this transition: (1) the nature of the border of the scalariform apertures and of the porous openings; (2) the number of the bars; (3) the width of the scalariform openings; (4) the inclination of the end walls; and (5) the relationship between the pitting of the end and side walls.

1. SCALARIFORM APERTURES.—These are of four types: with a complete border, with a border complete to the middle of the orifice, with a border only at the ends of the orifice, and non-bordered apertures. There are, from this point of view, only two types of porous

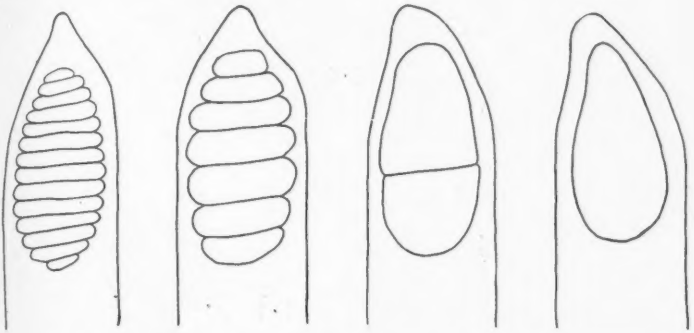


FIG. 1.—*Myrica inodora*, four stages showing origin of simple perforation

perforations which are significant. The openings may be completely to partially bordered or non-bordered.

The pits of the scalariform tracheid type, which gave rise to the primitive vessel segment of the dicotyledons, are invariably bordered. The loss of the membrane from a pit of this nature would result in an orifice with a complete border, and such being the case, one would expect that the most primitive type of scalariform opening would be completely bordered, and that the loss of these borders would be evidence of specialization. Table III shows the correlation between the nature of the border of the orifice and the length of the vessel segment.

The vessel segments of group I, as shown by their great average length, are considerably more primitive than the vessel segments of

group II. It follows that the completely bordered type is more primitive than the partially bordered or non-bordered type. Table IV illustrates how well the specialization of the length of the vessel segment, and thereby all characteristics associated with length, correlates with the specialization of the border of the openings of the scalariform perforation. In the primitive condition of the perforation the border completely surrounds the openings; in the second stage the border is complete to the middle of the apertures only; in the third stage the border has disappeared from the sides and remains

TABLE III

WOODS WITH SCALARIFORM PERFORATIONS

TYPE OF ORIFICE	NO. OF SPECIES	VESSEL SEGMENT LENGTH (MM.)
I. Completely bordered.....	16	1.34
II. Incompletely to non-bordered.	35	0.98

at the ends of the openings only; and in the final transformation the border is entirely lost.

It would be possible to increase the number of cases used in forming these averages by increasing the number of species. This would involve taking more than one species from each genus, and would give an apparent, not a real stability, to the differences between the means. In all cases the actual differences between the means are more than three times their probable errors.

A considerable number of species exist with dominantly bordered perforations in the vessels of the secondary xylem, but very few in which all the perforations are bordered. This is because the completely bordered scalariform opening is the most primitive, and the wood of existing dicotyledons, with the exception of that of *Tetracentron*, etc., is considerably specialized. *Dillenia philippinensis*, whose vessel segments average 1.6 mm., is a good example of a wood in which the orifices are dominantly bordered.

Species with perforations which have borders to the middle of the orifice or only at the ends of the openings do not exist as a pure type, since the completely bordered condition is present as a vestigial characteristic or because the next type in the sequence is being in-

troduced. The primitive type is lost by slow stages and the advanced type enters in the same way. This lack of rigid lines of distinction between specific characters is responsible for the preservation of sequences which reflect the evolutionary history of certain characterstics. In *Hamamelis virginiana*, whose vessel segments average 0.8 mm. in length, the entire sequence from the fully bordered to the non-bordered condition may be constructed from individual variability (figs. 2, 3).

The situation at the end of the sequence is somewhat different since no new type appears, and since there has been sufficient time

TABLE IV

WOODS WITH SCALARIFORM AND SCALARIFORM-POROUS PERFORATIONS

TYPE OF ORIFICE	NO. OF SPECIES	LENGTH OF VESSEL SEGMENT (MM.)
I. Completely bordered	19	1.27
II. Bordered to middle	16	1.07
III. Bordered only at ends	38	0.82
IV. Non-bordered	32	0.57

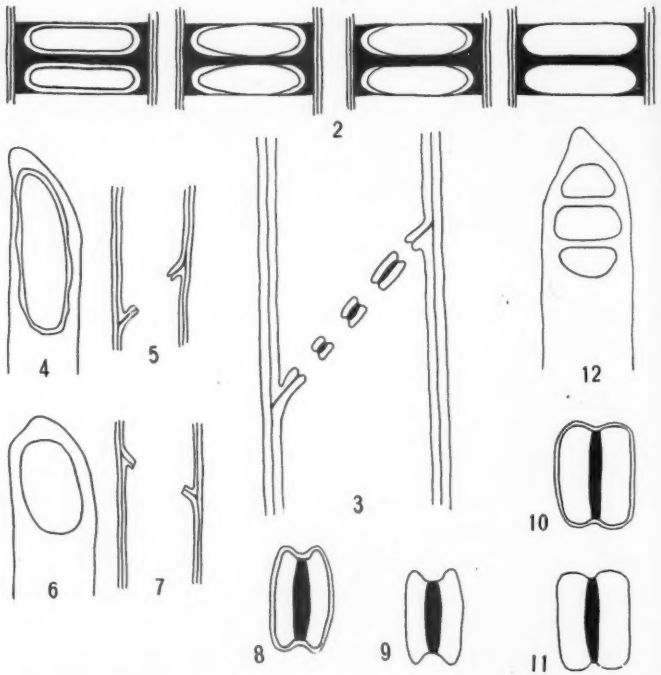
for the primitive types to disappear. *Alnus rubra*, whose average vessel segment length also is 0.8 mm., is an illustration of a type in which practically all of the perforations are non-bordered.

That the bordered porous opening is more primitive than the non-bordered (figs. 4, 5) is indicated by the fact that the former condition is common in scalariform-porous woods and rare in transverse porous woods, whereas the latter condition is rare in scalariform-porous woods and characteristic of transverse porous woods.

Since the length of the vessel segments of the dicotyledons is correlated with the type of perforation and also with the type of border of the apertures of the perforation, it follows that the type of border must be correlated with the type of perforation. This correlation is given in table V.

In addition to showing the correlation between the type of border and the type of perforation, table V shows several other points of interest. There are evidently two major lines of phylogenetic spe-

cialization, considering the relationships of the rates of specialization of the border of the aperture and the perforation as a whole: (*a*) the completely bordered to non-bordered transition may be concluded before the introduction of the porous perforation; and (*b*) the porous perforation may be introduced before the border transition is fairly started.



FIGS. 2–12.—Fig. 2, *Hamamelis virginiana*, four stages in evolutionary loss of borders from scalariform apertures; fig. 3, *Hydrangea peruviana*, bordered scalariform perforation in section; figs. 4, 5, *Sambucus callicarpa*, bordered porous opening in surface and sectional view; figs. 6, 7, *Ehretia elliptica*, non-bordered porous opening in surface and sectional view; figs. 8, 9, *Viburnum tinoides*, completely bordered bar showing extra layer of wall substances; figs. 10, 11, *Alnus rubra*, slightly bordered bar showing same condition; fig. 12, *Paeonia moutan*, example of perforation with only a few bars.

(*a*) The first line of specialization is illustrated by the following diagram:

I		II		III		IV	V
Completely bordered Scalariform	→	Bordered to middle Scalariform	→	Bordered at ends Scalariform	→	Non-bordered Scalariform	Non-bordered Porous

This is the only sequence which will account for scalariform woods with scalariform apertures bordered to the middle, bordered at the ends, and non-bordered. The number of scalariform species in groups I and II of table V is high relative to the number of scalariform-porous species, indicating that this part of the sequence is the

TABLE V*

Type of border	Percentage entirely scalariform (primitive)	Percentage scalariform-porous (intermediate)
I. Complete (primitive)	89	11
II. Complete to middle (intermediate)	64	36
III. Bordered at ends (intermediate)	56	44
IV. Non-bordered (specialized)	50	50

* Based upon a study of 108 species.

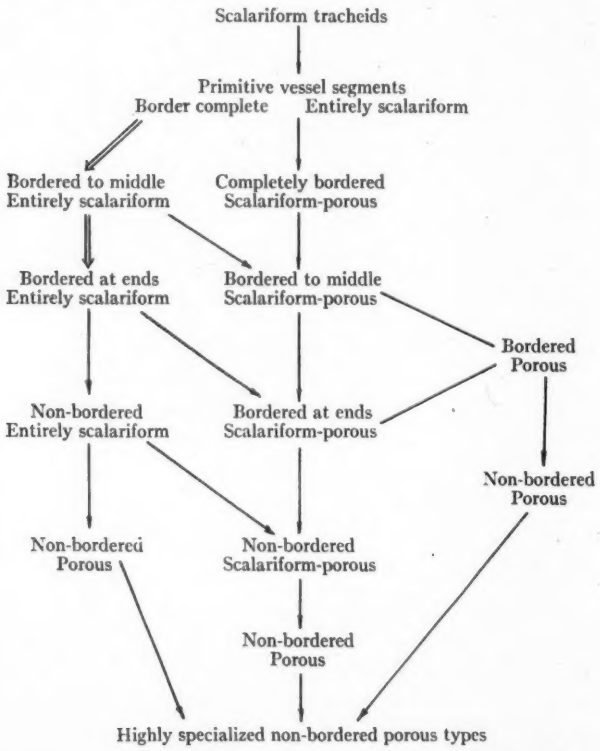
generally adopted pathway to the transverse porous perforation. The non-bordered scalariform perforation can give rise only to the non-bordered porous perforation, and therefore the bordered porous type could not have arisen from this sequence.

(*b*) The second line of specialization is more complicated, since offshoots of the first series may give rise to types in the second series. The main line is as follows:

I		II		III		IV	V
Completely bordered Scalariform	→	Completely bordered Scalariform-porous	→	Bordered to middle Scalariform-porous	→	Bordered at ends Scalariform-porous	Non-bordered Scalariform-porous

This transition accounts for the scalariform-porous types which are dominantly bordered, and perhaps for the remaining types, although the others may be modifications of the major line of specialization.

The following diagram illustrates all the possibilities.



It will be noted that the bordered porous condition, which is not uncommon, can arise only from stages III and IV in the scalariform-porous transition. It is also probable that the step from the scalariform completely bordered type to the scalariform-porous completely bordered type is of rare occurrence. The bordered to middle scalariform-porous stage generally arises from the bordered to middle scalariform stage. This is indicated by the fact that the proportion of types I and II is higher in the scalariform series than in the scalariform porous series. Fundamentally, of course, the two major lines just given are the same, since they are differentiated only on the basis of the time of introduction of the porous perforation.

The membranes of a scalariform perforation, in the normal course of events, are lost after the cell has completed its ontogeny. The writer, however, has noticed both bordered and simple bars which are completely encircled by an additional layer or wall, which must have been deposited after the absorption of the membranes. These curious bars occur at random in all types of scalariform perforations and are of no evolutionary significance. Two varieties are illustrated in figs. 8–11.

2. NUMBER OF BARS.—The bars composing a scalariform perforation may be many, intermediate, or few in number. If it is true that the scalariform tracheid gave rise to the vessel segment the

TABLE VI

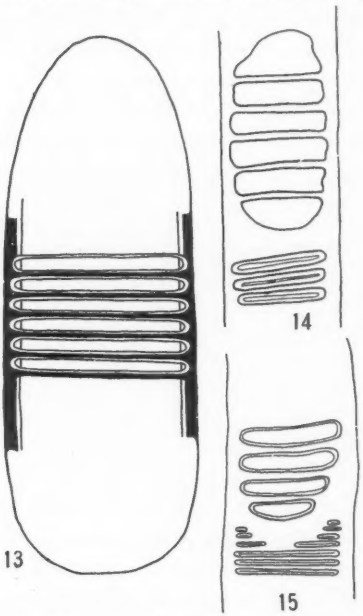
TYPE OF BORDER	SPECIES			PERCENTAGE OF MANY + INTERMEDIATE WITH FEW-BARRED TYPE
	Many	Intermediate	Few	
I. Completely bordered...	8	6	4	77
II. Bordered to middle....	7	4	5	69
III. Bordered at ends.....	6	19	13	65
IV. Non-bordered.....	0	14	18	43

many-barred type should be primitive, since the apertures of scalariform pits of tracheids are narrow and since the plane of contact between tracheids, in approximate vertical series, is long. The loss of pit membranes from a common wall of this nature would result in a many-barred perforation.

Defining roughly the many-barred type as over fifteen, the intermediate type as five to fifteen, and the few-barred type as five or less, table VI shows their correlation with the bordered to non-bordered sequence.

It is seen from table VI that the number of bars decreases with increasing specialization of the perforation, indicating that the many-barred type is primitive and the few-barred type is specialized. It is also pertinent to note that vestigial scalariform perforations are always characterized by a small number of bars. *Gordonia lasianthus* is an illustration of the many-barred condition, and *Paeonia moutan* is an excellent example of extreme reduction in bar number (fig. 12).

After the number of bars has been reduced to a very few, further specialization generally results in a loss of the remaining bars *in toto*, since intergrading forms, from the few-barred to the porous type, are rather unusual. This complete dropping out of the membrane of the perforation occasionally occurs at an earlier stage in the evolutionary development of the scalariform perforation. *Urandra luzoniensis* is an example of this variation. In this species the normal scalariform perforations are primitive and have numerous bars. Porous perforations are also present but the normal intermediate types are lacking. That the porous condition arises from the sudden loss of bars is indicated by the fact that perforations are common which are scalariform with many bars on one side of the membrane and porous on the other side of the membrane (fig. 13).



FIGS. 13–15.—Fig. 13, *Urandra luzoniensis*, showing occurrence of perforation which is scalariform on one side of membrane and porous on other; fig. 14, *Telauma ovalis*, comparison of width of scalariform apertures with width of apertures of scalariform lateral pits; fig. 15, *Bruguiera parviflora*, same as fig. 14.

With increasing specialization of the vessel segment, as will be shown later, the end wall changes from a highly inclined to a transverse position, resulting in a considerable decrease in the length and area of the end wall. If the number of bars remained constant during this process they would gradually be crowded together and the apertures reduced in size. This would therefore result in a positive correlation between the resistance against the passage of a fluid and the phylogenetic development of the vessel. On the other hand, if the number of bars decreases faster than is necessary to maintain

a constant width of aperture, the resistance to flow will decrease with the specialization of the vessel segment. It becomes evident, then, since the apertures do not decrease in size, that this line of specialization is advantageous to the ready flow of a liquid through the vessel. Whether this is advantageous to the plant as a whole is of course a different question, but one which might yield interesting results if tested experimentally.

Fig. 1, drawn from *Myrica inodora*, illustrates how the reduction in the number of bars is reflected in irregular variability in space in the secondary xylem of this species. (Note also figs. 6–10 of the first paper of this series.)

3. WIDTH OF SCALARIFORM OPENINGS.—In both the transitions referred to the width between the bars increases with specialization of the vessel segment. This certainly results in a decrease in resistance to flow and is accomplished by two factors which work in conjunction. The first cause which increases the width of the apertures is the loss of borders. It is not surprising that the borders disappear, since there seems to be no reason, other than the fact that the openings originated from scalariform pits, for their existence. When present, the borders partially fill the openings and increase the resistance to the passage of a fluid. The second and most important cause, which enlarges the openings of the scalariform perforation, is an actual reduction in the number of bars at a more rapid rate than that needed to maintain a constant size of aperture. *Talauma ovalis* and *Bruguiera parviflora* are excellent examples of the widening process. In *Talauma* the vessel segments are short and the borders often absent. The scalariform pitting of the side walls, however, has remained practically unchanged from the primitive condition. A comparison of the apertures of the side and end wall indicates the amount of phylogenetic widening of the openings of the scalariform perforation (fig. 14). In some instances the scalariform openings are more than ten times as wide as the apertures of the lateral pits. *Bruguiera parviflora* is a more primitive species with longer vessel segments. In this species the completely bordered condition is common, and in such cases the widening of the openings is due solely to a reduction in the number of bars (fig. 15).

It must be remembered that the actual width of the openings of

a scalariform perforation is correlated, to some extent, with the size of the vessel segment. In making comparisons of this characteristic, therefore, the size of the vessel segment must be taken into account. It is also true that the openings may increase only slightly during specialization of the vessel segment, and for this reason the wide opening is a much better indication of specialization than the narrow opening is of primitiveness.

4. INCLINATION OF END WALLS.—There is a strong correlation between the type of perforation and the inclination of the end wall. The primitive scalariform perforation is generally oblique, and the specialized porous perforation nearly always transverse. Table VII indicates the trend of this development.

TABLE VII

BASED ON 104 SCALARIFORM AND SCALARIFORM-POROUS WOODS

TYPE OF PERFORATION	PERCENTAGE SPECIES WITH HIGHLY INCLINED END WALLS
I. Border complete	73
II. Partially bordered	41
III. Non-bordered	13

Table I shows that the porous species with oblique end walls are more primitive than the porous species with transverse end walls. The porous-oblique perforations are caused by a lag in the rate of change of the position of the end wall in relation to the time of introduction of the porous perforation. That is, if the change from scalariform to porous perforation occurs before the end wall has reached the transverse condition, an oblique porous perforation will result. That this is the normal case is shown by the great preponderance of oblique porous end walls in scalariform-porous species.

If the assumption is made[2] that the vessel segment is frequently subjected to collapsing pressures, due to tension of the liquid column, specialization of the end wall is productive of greater rigidity. A brace parallel to the direction of force, such as the transverse end wall, is better adapted to resist crushing forces than an

[2] In accordance with H. H. DIXON's theory of the ascent of sap.

oblique brace, and in addition the shortening of the vessel segment produces a greater number of braces per unit of length. It is not impossible to suppose that the huge vessels could not have evolved, in regions of high transpiration, if the vessel segment had not shortened in length and the perforation become transverse.

5. PITTING OF END AND SIDE WALLS.—BROWN (3) contends that the rate of specialization of the end wall would be slower than the

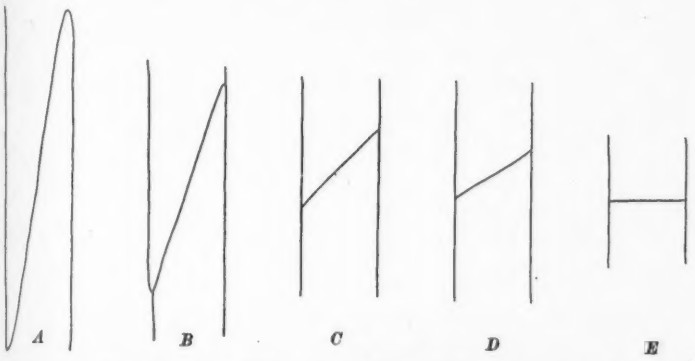


FIG. 16.—Evolution of inclination of end wall: (a) *Saurauja oldhami*, (b) *Panax edgerlegi*, (c) *Gilbertia affinis*, (d) *Diospyros virginiana*, (e) *Hicoria glabra*.

rate of specialization of the side wall since the end wall always separates tracheary elements, whereas the side wall comes into contact with a variety of cell types. In 51 entirely scalariform species, 29 have both scalariform side and end walls, while 22 show a distinct change in pitting from the end to the side wall. In 45 scalariform-porous species only four have scalariform lateral walls. These data would indicate that BROWN is correct. While these rates are undoubtedly of different magnitude, it must be remembered that slightly different rates will produce different results only after acting over a period of time, and this explains why the majority of scalariform woods have vessel segments with scalariform lateral pitting.

The occurrence and development of the reticulate, multiperforate, etc., types of perforations are not discussed in this paper, since the matter has been covered in detail by THOMPSON (7).

Summary

1. The scalariform perforation is primitive and the porous perforation is specialized. The phylogenetic order of development is: scalariform, scalariform-porous, oblique porous with vestiges of the scalariform condition, and transverse porous.

2. The primitive fully bordered aperture of a scalariform perforation gradually loses its border as the perforation becomes specialized.

3. The primitive scalariform perforation has many bars, which are generally lost in slow stages in correlation with the evolutionary development of the perforation.

4. Specialization of the scalariform perforation frequently results in a widening of the apertures of the perforation.

5. The inclination of the end wall changes from the highly inclined position to the transverse position as the scalariform perforation develops into the porous perforation.

6. The lateral pitting specializes more rapidly than the specialization of the perforation.

The writer wishes to take this opportunity to thank Professor I. W. BAILEY for his continued advice and criticism.

CUMBERLAND MILLS
MAINE

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MICROSPOROGENESIS IN THE CUCURBITACEAE

SARA F. PASSMORE

(WITH FORTY FIGURES)

Comparatively little work has been done on the development of the pollen mother cell of members of the Cucurbitaceae. STRASBURGER (10), in work on the determination of sex, refers to conditions in *Bryonia alba;* BOENICKE (2) and LUNDEGARDH (8), in their work on the heterotypic cell division, have studied members of this group, the former, *Bryonia dioica*, and the latter, *Cucurbita pepo*. *B. alba* and *B. dioica* have been studied by MEURMAN (9) in his work on sex chromosomes. None of these workers has given a detailed account of microsporogenesis. KIRKWOOD (6), however, has fully described the process in *Micrampelis lobata*. CASTETTER (3) has described it in *Cucurbita maxima*, and HEILMICH (5) in *Cucumis sativus*. KOZHUCHOW (7), although his work has been chiefly on the vegetative cells, studied the chromosomes of *Citrullus vulgaris*, *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *C. moschata*, and *C. pepo*. The reported chromosome numbers in the Cucurbitaceae are listed in table I.

Materials and methods

The following commercial varieties, arranged according to BAILEY'S (1) classification, were obtained from Dreer and Company, Seedsmen:

Cucurbita pepo	Jersey White Bush squash
Cucurbita pepo	English vegetable marrow
Cucurbita maxima	Warted Hubbard squash
Citrullus vulgaris	Kleckley Sweets watermelon
Luffa cylindrica	Luffa gourd
Cucumis melo	Rockyford cantaloupe
Cucumis sativus	White Spine cucumber

The plants were grown in the field, and fixations of the staminate buds were made at different hours of the day and night. Fixations made between 7:00 and 8:00 A.M. yielded more reduction division figures than those made at other hours, although cells in hetero-

typic division were found at other times also. Buds 1–8 mm. in length were fixed, the 5 mm. ones proving most fruitful in the plants of *Cucurbita*. In all other genera studied, buds about 2 mm. long contained cells in reduction division stages. The perianths were removed in the larger buds. The smaller ones were cut longitudinally with a sharp knife before being placed in the fixative.

TABLE I

REPORTED* CHROMOSOME NUMBERS IN THE CUCURBITACEAE

SPECIES	1n CHROMOSOMES	2n CHROMOSOMES
Bryonia alba	10 (Boenicke, Meurman)	.....
Bryonia dioica	10 (Strasburger, Meurman)	.....
Citrullus vulgaris	11 (Passmore)	22 (Kozhuchow, Passmore)
Cucumis melo	12 (Passmore)	24 (Kozhuchow)
Cucumis sativus	7 (Heimlich)	14 (Kozhuchow, Heimlich, Passmore)
Cucurbita maxima	20 (Castetter, Passmore)	40 (Castetter)
Cucurbita maxima	.....	44–48 (Kozhuchow)
Cucurbita moschata	.....	48 (Kozhuchow)
Cucurbita pepo	.....	24 (Lundegardh)
Cucurbita pepo var. pomiformis	.....	40 (Kozhuchow)
Cucurbita pepo var. citrullina	.....	42 (Kozhuchow)
Cucurbita pepo (Jersey White Bush)	20 (Passmore)	.....
Cucurbita pepo (English vegetable marrow)	20 (Passmore)	.....
Luffa cylindrica	11 (Passmore)	.....
Micrampelis lobata	16 (Kirkwood)	.....

* See Literature Cited at end of paper for references.

Various modifications of Flemming's, Bouin's, and Benda's solutions were tried, and the following Flemming type solution seemed most valuable: chromic acid (10 per cent) 0.40 cc., acetic acid (10 per cent) 4.00 cc., osmic acid (2 per cent in 2 per cent chromic) 3.00 cc., water 16.60 cc., maltose 0.3 gm. The Benda type solution which gave exceptionally good results in a few cases, but on the whole did not seem as dependable for the material as the preceding, was: 1 per cent chromic acid 16 cc., 2 per cent osmic acid 4 cc., glacial acetic acid, 2 drops.

The material was imbedded in paraffin, and sections from 7 to 20 μ in thickness were cut and stained with Haidenhain's iron-alum

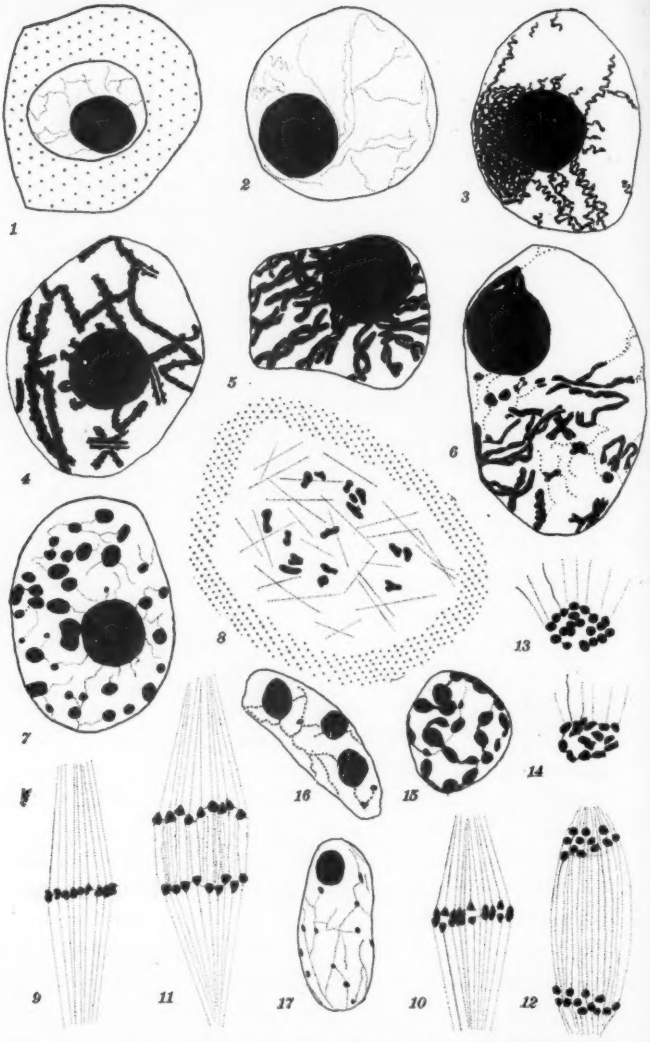
haematoxylin. Drawings were made with the help of the camera lucida.

This paper describes the development of the pollen mother cell of *Cucurbita pepo* (Jersey White Bush squash) from presynizesis to the quartet stage, and also shows the chief differences noted in microsporogenesis of the other varieties.

Cucurbita pepo

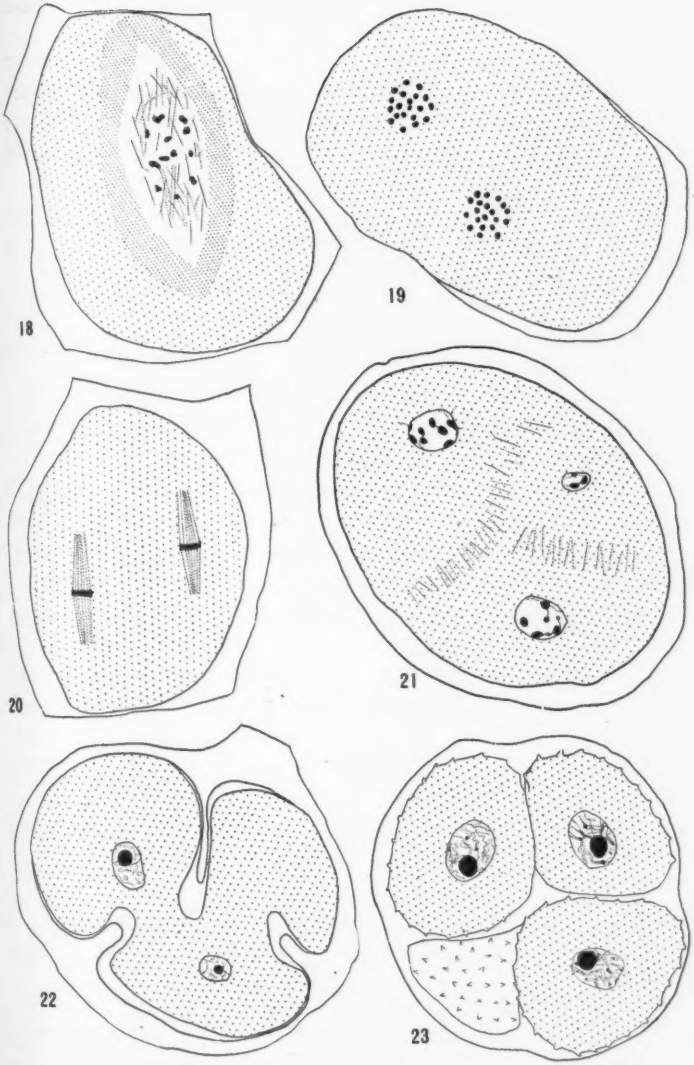
The cytoplasm in the primary sporogenous cells, which become the spore mother cells, is very dense. The nucleus contains a large deeply staining nucleolus, but the chromatin is rather meager and stains only faintly (fig. 1). Both the nucleus and the cell enlarge rapidly. The leptotene threads show some tendency to pair (fig. 2), but it is impossible to tell whether the apparent pairing which occurs here and there is significant or simply accidental. The spireme still stains rather faintly, but its staining property increases as it enters synizesis (fig. 3). When the spireme emerges from synizesis it has a beaded appearance and its doubleness shows plainly. It already seems to have divided transversely into segments of various lengths (fig. 4). The double segments twist themselves in ropelike fashion, and the beaded appearance becomes lost (fig. 5). At this stage the nucleolus is at its maximum size, but there seems to be a tendency for the nucleus as well as the cytoplasm to contract. The segments soon loosen and lie scattered through the nucleus (fig. 6), each apparently breaking into several parts (fig. 7). The nucleolus by this time is much reduced in size. It is at this stage of development of the nucleus that the dense perinuclear zone so conspicuous at metaphase first makes its appearance.

Fig. 24 shows the condition of the nucleus just before the membrane disappears. The pairs of chromosomes lie scattered through the nucleus, and among them are many darkly staining bodies. The nucleolus has disappeared at this time. When the nuclear membrane disappears, the spindle fibers, which seem to be partly of nuclear and partly of cytoplasmic origin, appear among the pairs of chromosomes (fig. 8). From this multipolar stage the spindle gradually becomes bipolar (fig. 18), and the pairs of chromosomes are arranged at the equator (fig. 9). A polar view at metaphase shows



FIGS. 1–17.[1]—Fig. 1, young pollen mother cell; fig. 2, nucleus showing leptotene threads; fig. 3, synizesis; fig. 4, pachynema, segments showing doubleness; fig. 5, strepsinema, chromosomes twisted about one another; fig. 6, early diakinesis; fig. 7, late diakinesis; fig. 8, multipolar spindle with double chromosomes in meshes; fig. 9, first metaphase; fig. 10, end of metaphase, homologous chromosomes separating; fig. 11, anaphase showing attenuated form of chromosomes as they pass to poles; fig. 12, late anaphase; fig. 13, twenty chromosomes at pole of spindle; fig. 14, early telophase (chromosomes have lost rounded appearance); fig. 15, nuclear membrane formed at telophase, chromatin and nucleoli reorganizing; fig. 16, late telophase showing three nucleoli; fig. 17, nucleus at end of first division.

[1] All figures are of Jersey White Bush squash, ×1500.



FIGS. 18–23.[2]—Fig. 18, spindle becoming bipolar, paired chromosomes arranging themselves at equator; fig. 19, polar view of second metaphase; fig. 20, second metaphase, spindles parallel; fig. 21, late second telophase; fig. 22, furrows dividing cell into quartets; fig. 23, microspores within sporocyte wall.

[2] All figures are of Jersey White Bush squash, except fig. 19, which is English vegetable marrow; ×750.

twenty bivalent chromosomes (fig. 25). KOZHUCHOW (7) reports forty for the vegetative cells of *Cucurbita pepo* var. *pomiformis* and forty-two for var. *citrullina*. LUNDEGARDH (8) reports twenty-four for the vegetative cells, a number which TISCHLER (11) thinks doubtful.

The homologues separate and pass to the poles with no evidence of lagging (figs. 10–12). The spindle attachments give the chromosomes an attenuated appearance as they move apart. Soon after the chromosomes reach the poles (fig. 13) they lose their rounded appearance and become angular and elongated (fig. 14). The nuclear material assumes a linklike appearance (fig. 15) in the reorganization of chromatin and nucleoli, as the membrane forms at telophase. Several nucleoli are formed at first (fig. 16), but usually only one is present at the end of the first division (fig. 17). This stage is quickly followed by second metaphase. The two spindles may be parallel (fig. 20) or they may occupy various other planes. Fig. 21 shows the pollen mother cell in second telophase. Division into quarters takes place by furrowing. Quadripartition is simultaneous in most cases (fig. 22). Occasionally furrowing seems to be more rapid in one plane than in the other, so that the cell is divided into two parts before complete quadripartition takes place. The microspores develop their characteristic markings while still inside the sporocyte walls (fig. 23).

During development of the pollen mother cell considerable growth takes place in the protoplast. The diameter of the protoplast in four stages of development was obtained by averaging the maximum and minimum diameters of five cells. The result was as follows:

Archesporium	Synizesis	First metaphase	Second telophase
24μ	48μ	58μ	65μ

This method of obtaining the size of the cells is not strictly accurate, but at least gives an idea of the great change in size that takes place.

The development of the pollen mother cell in the English vegetable marrow is identical with that of the other variety of *Cucurbita pepo*, so far as the writer could ascertain. The chromosome number is likewise twenty (fig. 19).

CUCURBITA MAXIMA

This species seems also to be identical in its development of the pollen mother cell with that of *Cucurbita pepo* just described. CASTETTER (3) described microsporogenesis in this species, from the development of the spindle to the quartet stage. The writer's material seems to be in agreement with his observations, except that he reports cell plates which were not observed in this material. The haploid chromosome number is twenty (fig. 26). KOZHUCHOW, working on the root tips of *C. maxima*, reports 44–48 chromosomes as the diploid number. One of the difficulties in work of this kind, however, lies in establishing the fact that varieties and species that are given the same name are actually identical.

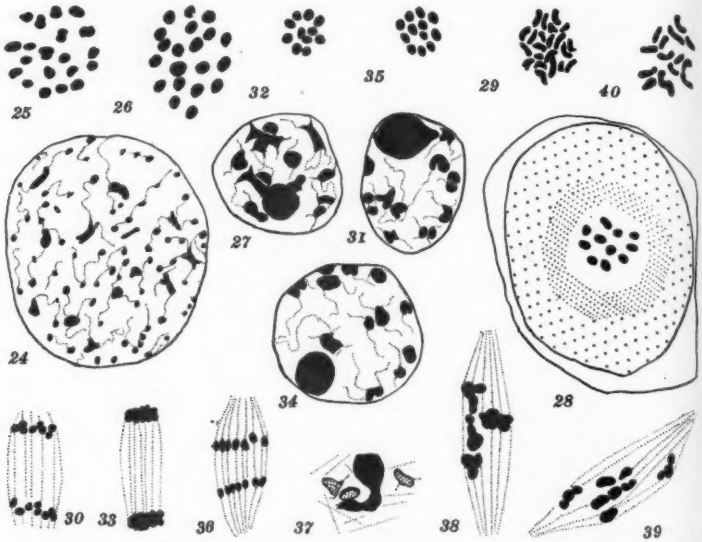
CITRULLUS VULGARIS

In all stages of development, the pollen mother cells of this and of the plants that follow are much smaller than those of *Cucurbita*. A cell at metaphase in this genus is only about one-fourth the size of the cell in the corresponding stage in *Cucurbita*. So far as the writer could ascertain, development up to diakinesis proceeds as in *Cucurbita pepo*. Here the paired chromosomes (fig. 27) can be seen lying about the nucleus, but there are no extra darkly staining granules among them, as in *C. pepo*. The nucleolus remains intact until the membrane disappears. A polar view at metaphase shows eleven bivalent chromosomes (fig. 28). Root tips show twenty-two chromosomes (fig. 29), the same number reported by KOZHUCHOW. At anaphase the chromosomes separate promptly with no evidence of lagging (fig. 30).

LUFFA CYLINDRICA

The chief difference noted in the development here is that at diakinesis the bivalent chromosomes scattered through the nucleus (fig. 31) lack the darkly staining bodies so evident in *Cucurbita pepo*. The nucleolus shows a tendency to bud, but is intact until the membrane disappears. It is sometimes found among the spindles at the multipolar stage, at which time, however, it is much reduced in size. There are eleven bivalent chromosomes (fig. 32), the number apparently not having been reported before. At anaphase the homologues separate and pass promptly to the poles with no evi-

dence of lagging (fig. 33). The appearance of the chromosomes in *Luffa* is similar to that in *Citrullus*. It is interesting to note in this connection that the seeds of *Luffa* are almost the same as those of *Citrullus* in size and markings. The pollen grains of the two are also practically identical.



FIGS. 24–39.[1]—Fig. 24, *Cucurbita pepo:* late diakineses showing paired chromosomes among other darkly staining bodies; fig. 25, same: polar view of first metaphase showing twenty bivalent chromosomes; fig. 26, *Cucurbita maxima:* polar view of second metaphase showing twenty chromosomes; fig. 27, *Citrullus vulgaris:* late diakineses showing eleven paired chromosomes; fig. 28, same: polar view of pollen mother cell at first metaphase showing perinuclear zone and eleven chromosomes; fig. 29, same: polar view of root tip cell showing twenty-two chromosomes; fig. 30, same: first anaphase; fig. 31, *Luffa cylindrica:* diakinesis showing ten of the eleven pairs of chromosomes, nucleolus budding; fig. 32, same: polar view of first metaphase showing eleven chromosomes; fig. 33, same: late anaphase; fig. 34, *Cucumis melo:* diakinesis, twelve pairs of chromosomes; fig. 35, same: polar view of first metaphase showing twelve chromosomes; fig. 36, same: first anaphase; fig. 37, *Cucumis sativus:* multipolar stage showing paired chromosomes among spindle fibers, nucleolus budding; fig. 38, same: chromosomes arranged irregularly on spindle; fig. 39, same: spindle showing two trivalent chromosomes passing to poles; fig. 40, same: polar view at metaphase in root tip cell showing fourteen chromosomes.

[1] All figures are ×1500, except fig. 26, which is ×2130.

Cucumis melo

The pollen mother cell of *Cucumis melo* at diakinesis (fig. 34) has much the appearance of that of *Citrullus* and *Luffa*. Darkly staining granules are not present among the pairs of chromosomes. The nucleolus disappears with the disappearance of the membrane in all cases noted. Polar views at metaphase show twelve bivalent chromosomes (fig. 35). Kozhuchow reports twenty-four for the root tip cells. There is no evidence of lagging as the chromosomes pass to the poles at anaphase (fig. 36).

Cucumis sativus

Many of the pollen mother cells in the anthers of this species degenerate before diakinesis. About half of the contents produce pollen that looks normal, although none of it was tested for fertility. At diakinesis the nucleolus is very prominent and stains deeply. The chromosomes stain faintly, but can be seen as irregular masses lying about the nucleus. At the time the nuclear membrane disappears and the spindle fibers appear, the nucleolus loses its rounded appearance and sends out tubelike projections (fig. 37). The irregular projections of the nucleolus are often difficult to distinguish from chromosomes, since the latter by this time also stain deeply. Heimlich states that the nucleolus of *Cucumis sativus* "becomes much elongated or verniform." He mentions the fact that the disappearance of the nucleolus is associated with the chromosomes at the multipolar stage. He also states that on account of the sizes and shapes of the elongations of the nucleolus, parts of it may be mistaken for chromosomes. As the spindle becomes bipolar the irregularly shaped masses of chromatin are distributed along it (fig. 38). There seems to be a tendency for some of the chromosomes to assume a trivalent formation (fig. 39), such as Darlington (4) figures for certain hybrid species of *Prunus*. The chromosome count could not be ascertained definitely in the pollen mother cell of this material. Heimlich reports seven chromosomes; root tip counts show fourteen (fig. 40), the number given by both Heimlich and Kozhuchow for vegetative cells. Certain cells in the periblem have twenty-eight chromosomes. In many of the root tip cells late wall

formation occurs, so that two nuclei frequently appear in the same cell. The nucleolus in root tip cells disappears by budding in much the same manner as in the pollen mother cell.

Summary

1. Darkly staining granules are present among the bivalent chromosomes in late diakinesis in all members of *Cucurbita*, but are absent in *Citrullus*, *Luffa*, and *Cucumis*.

2. *Cucurbita pepo* and *C. maxima* each has twenty bivalent chromosomes.

3. *Citrullus vulgaris* has eleven bivalent chromosomes; *Luffa cylindrica*, eleven; *Cucumis melo*, twelve. *Cucumis sativus* has fourteen chromosomes in the diploid cells.

4. The homologous chromosomes pass to the poles at anaphase with no evidence of lagging in *Cucurbita pepo*, *C. maxima*, *Citrullus vulgaris*, *Luffa cylindrica*, and *Cucumis melo*.

5. Chromosomes are distributed irregularly on the spindle at anaphase in *Cucumis sativus*.

6. The nucleolus shows a tendency to bud in both *Luffa cylindrica* and *Cucumis sativus*, and is often present after the spindle fibers appear in these species.

I wish to express appreciation to Professor W. R. TAYLOR for his interest and assistance in this work.

UNIVERSITY OF PENNSYLVANIA

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THE MICROFLORA OF LEACHED ALKALI SOIL

J. E. Greaves and J. Dudley Greaves[1]

Introduction

It is generally recognized that a soil to which a soluble salt has been added is not restored to its original condition merely by leaching. There have resulted far reaching physical, chemical, and biological changes in the properties of the soil, and in many cases it is with difficulty, if at all, that it can be restored approximately to its native condition. Moreover, it has been demonstrated that the microflora of a soil can be profoundly modified both qualitatively and quantitatively by the use of soluble salts, the extent depending upon the specific salt, its concentration, and the bacterial activity of the original soil. Some soluble salts in comparatively low concentration greatly retard or even check beneficial bacterial processes. Many soluble salts when in soils in low concentration greatly accelerate some beneficial bacterial activities. This may be due to their action upon the structure of the soil or the liberation of essential nutrients, and probably less often to a direct stimulating action of the salt upon the bacterial protoplasm. Hence the microflora of an alkali soil should be far different from that of a non-alkali soil.

Some soils, even after leaching, remain barren or produce only meager crops for a number of years. The non-productive period may be due to physical, chemical, or biological changes which have occurred within the soil owing to its alkali content or the changes incidental to the leaching process. It is not unreasonable to expect the microflora to be likewise greatly influenced, and it would require considerable time after the leaching of such a soil before a normal active microflora would become established. This non-productive period may be shortened by proper inoculation with productive soil.[2]

In order to gain more precise information on the microflora of leached alkali soil, during the past few years rather extensive studies

[1] Contribution from Department of Bacteriology and Chemistry, Utah Agricultural Experiment Station. Publication authorized by Director June 13, 1929.

[2] Greaves, J. E., The influence of salts on bacterial activities of soil. Bot. Gaz. 73:161–180. 1922.

have been conducted upon the specific microorganisms and their activity in leached alkali soil. Numerous studies have been made on various alkali soil, both naturally occurring and synthetically prepared, but the results reported in this paper were obtained on three soils: (1) a synthetic alkali soil made non-productive by the addition of 0.66 per cent each of sodium chloride, sodium sulphate, and sodium carbonate; (2) a naturally occurring alkali soil rich in chlorides and containing comparatively small quantities of the other soluble salts; (3) a natural occurring alkali soil rich in sulphates and containing only comparatively small quantities of other soluble salts. All three soils contained sufficient soluble salts to render them barren to all vegetation except a few salt-tolerant plants.

Investigation

The soils were packed into 2-gallon jars provided with a $\frac{1}{2}$-inch hole in the bottom for leaching. They were then permitted to stand

TABLE I

COLONIES DEVELOPING UPON SYNTHETIC AGAR FROM LEACHED ALKALI SOIL

SOIL	COLONIES DEVELOPING IN 7 DAYS		
	Just after leaching (thousands)	After crop of crimson clover (thousands)	After crop of barley (thousands)
Native	2573	3300	2250
Synthetic alkali	3042	10,250	2600
Synthetic alkali, leached	7200	9450	2650
Chloride	220	600	1800
Chloride, leached	885	3150	5450
Sulphate	1452	6000	3550
Sulphate, leached	1722	5050	3000

2 months with a moisture content of 20 per cent so that the soil would become packed and the various reactions tend toward equilibrium, after which they were leached for a period of 2 years, that is, until most of the salts which could be washed out by mere leaching were removed. They were planted after leaching to crimson clover and later to barley. Before each cropping the soils were sampled and analyzed as to numbers, ammonifying, nitrifying, and nitrogen-fixing powers. Finally the specific microflora which develop on synthetic Ashby media were studied in detail.

The addition of soluble salts to a calcareous loam and then the

leaching out of these salts greatly increased the number of microorganisms obtained from this soil by the plate method. Apparently the soluble salt and the water had removed some substance from the soil which keeps in check its natural microflora;[3] however, there is a direct stimulation of the bacteria by the remaining salts. This probably plays a major rôle in the change in numbers, but the results as a whole show the inadequacy of numbers as determined by the plate method for evaluating the productivity of soils. The ammonifying powers of the soil are more valuable for this purpose.

TABLE II

MILLIGRAMS OF AMMONIA PRODUCED IN 4 DAYS IN VARIOUS ALKALI SOILS CONTAINING 1 PER CENT DRIED BLOOD

SOIL	MG. AMMONIA PRODUCED IN 4 DAYS		
	Directly after leaching	After crop crimson clover	After crop barley
Native	89	91	66
Synthetic alkali	19	10	34
Synthetic alkali, leached	64	67	73
Chloride	2	2	5
Chloride, leached	40	16	57
Sulphate	11	11	50
Sulphate, leached	24	29	66

The addition of 0.66 per cent each of sodium chloride, sodium sulphate, and sodium carbonate to this highly productive calcareous loam reduced its ammonifying powers three-fourths, and leaching the soil brought it back to 80 per cent normal with a tendency for the ammonifying powers to increase after cropping.

The chloride-rich soil produced very small quantities of ammonia but after leaching produced 41 per cent of that produced by the check productive soil. The sulphate-carrying soil was producing more ammonia than the chloride-carrying soil, with an average of 24 mg. of ammonia. After leaching it was producing only 49 per cent of normal. It is evident, therefore, that during the time these soils were under observation, three years, the ammonifying powers of leached alkali soils did not reach that occurring in normal productive soils. It was found that it could be brought to normal, however, by the addition of soil extracts, farm manures, and plant residues. The

[3] GREAVES, J. E., Soil microbial stimulants. Proc. Ist International Congress Soil Science 1927. 3:222–228. 1928.

effect upon the nitrifying powers of the soils is even greater than upon the ammonifying powers.

At first the nitrifying power of the synthetic alkali soil was extremely low. Later, due to increased aeration, it became more active. The leaching of this soil not only restored its natural nitrifying powers but acted as a stimulant. It is evident that 0.66 per cent each of sodium chloride, sodium sulphate, and sodium carbonate is not sufficient to destroy the nitrifying bacteria, for when the salts are

TABLE III

MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 21 DAYS IN VARIOUS ALKALI SOILS CONTAINING 1 PER CENT DRIED BLOOD

Soil	Mg. of nitric nitrogen produced in 21 days		
	Directly after leaching	After crop crimson clover	After crop barley
Native	40.6	56.3	53.2
Synthetic alkali	1.4	33.5	23.8
Synthetic alkali, leached	46.9	64.7	87.2
Chloride	4.9	2.8	7.0
Chloride, leached	2.1	13.3	7.7
Sulphate	9.1	17.5	16.0
Sulphate, leached	5.9	10.9	19.3

removed and as the physical properties of the soil improve, the bacterial activities increase. On the other hand, leaching of the natural alkali soil did not restore its nitrifying powers, as few more nitrates were produced in the leached than in the unleached soil. It is in cases such as this that soil inoculation is essential to restore the natural microflora.

Soluble salts especially influence nitrogen fixation, and all evidence indicates that this class of microorganisms are more resistant to soluble salts than are the other beneficial bacteria.[4]

The alkali-containing soil uniformly gained in nitrogen when incubated with a soluble carbohydrate. The extent to which this occurs depends upon the specific salt present. It is most active in the presence of sulphate and least active in the presence of chlorides. Good azotobacter membranes invariably appeared when the sulphate-containing soils were used as the inoculant. The synthetic alkali soil was much more active in fixing nitrogen both before and

[4] GREAVES, J. E., The microflora and the productivity of leached and non-leached alkali soil. Soil Sci. 23:271–302. 1927.

after leaching than was the native soil. When these soils were inoculated into Ashby media the nitrogen gains were even greater than they were in soil; consequently all results indicated that the alkali salts, if not present in too great concentration, accelerated nitrogen gains in soil. In order to obtain a more definite measure of the soil gains in nitrogen, they were carefully analyzed at the beginning of the experiment and at the end of two years.

TABLE IV

MILLIGRAMS OF NITROGEN FIXED IN 21 DAYS IN VARIOUS ALKALI SOILS CONTAINING 1.5 PER CENT LACTOSE

SOIL	MG. OF NITROGEN FIXED IN 21 DAYS IN SOIL		
	Directly after leaching	After crop crimson clover	After crop barley
Native	− 6.3	−0.8	10.5
Synthetic alkali	2.8	2.8	0.7
Synthetic alkali, leached	10.0	3.3	1.1
Chloride	4.1	0.9	1.8
Chloride, leached	0.0	1.8	0.3
Sulphate	.....	3.9	8.3
Sulphate, leached	.....	6.1	4.2

TABLE V

CALCULATED POUNDS OF NITROGEN GAINED BY 1 ACRE-FOOT OF SOIL DURING 2 YEARS

SOIL	DIRECTLY AFTER LEACHING (LB. PER ACRE)	END OF 2 YEARS (LB. PER ACRE)	TOTAL LOSS OR GAIN
Native	5397	4885	−512
Synthetic alkali	4536	4863	327
Synthetic alkali, leached	4385	5055	670
Chloride	4738	5073	335
Chloride, leached	4334	5104	770
Sulphate	10,764	11,052	298
Sulphate, leached	10,300	11,442	1142

These soils were kept in the greenhouse with an optimum moisture content. The water from the city mains was used: this is low in soluble constituents. There had been produced on these soils one crop of crimson clover and two of barley. In the case of the alkali-containing soils the yield was light. Roots, stocks, and grain were removed when harvesting; hence little nitrogen could have come from the legume crop, yet the alkali-containing soils invariably registered appreciable gains of nitrogen. In the case of the leached

soils this was considerable. The sulphate-containing soil made the greatest gains. There was an annual loss of nitrogen from the check soil. The synthetic alkali soil gained annually 163 pounds of nitrogen. The gain in the leached soil was twice this amount. The chloride-containing soil gained 167 pounds of nitrogen per acre annually, with a gain of twice this amount by the leached soil. The sulphate-carrying soil gained annually 150 pounds of nitrogen, with a gain of four times this amount by the leached alkali soil. It is evident, therefore, that the alkali salts are increasing the nitrogen-fixing powers of these soils. The question at this stage was: what organisms are there within the soil responsible for this gain in soil nitrogen?

After the second crop of barley was harvested the junior author sampled the three leached soils and made a careful study of their specific microflora. The soils were first plated on nutrient, synthetic, and Ashby agar. There was a wide variation in the number and kind of colonies which developed on these different media.

TABLE VI

COLONIES DEVELOPING ON DIFFERENT MEDIA FROM LEACHED ALKALI SOIL

SOIL	NUTRIENT AGAR	SYNTHETIC AGAR	ASHBY AGAR
Synthetic alkali, leached	4,505,000	4,567,000	2,808,000
Chloride, leached	938,000	450,000	5,350,000
Sulphate, leached	5,600,000	3,878,000	3,855,000

The number of colonies developing varied with the media and soil. The synthetic alkali soil yielded approximately the same number of colonies on the nutrient and synthetic agar, with fewer on the Ashby. The chloride-carrying soil yielded many more colonies on the Ashby than on either of the others, whereas the sulphate-carrying soil yielded the greatest number on the nutrient, and fewer but approximately the same number on synthetic and Ashby agar. It was evident, therefore, that these soils contained many microorganisms which would develop on a nitrogen-poor medium such as Ashby's. The different colony types developing on Ashby agar were obtained in pure culture by repeated dilution, plating, and microscopic examination. They were then studied in detail morphologically and physiologically, according to the Official Methods.

Sixty-eight organisms were obtained in pure culture, all but 7 of which were gram-positive. There were 39 bacilli, 16 cocci, and 13 filamentous. Spores were produced by approximately 50 per cent, while only one-fourth of the organisms were motile. Glucose was fermented by 37, and of these 8 fermented lactose and 15 sucrose. All but 14 hydrolized starch. Ninety per cent produced ammonia when grown in a 1 per cent peptone solution. The quantity produced varied from 1 to 38 mg. Six produced over 20 mg. and one produced between 10 and 20 mg. Thirty-eight fixed nitrogen when incubated for 6 weeks in sterilized soil. The quantity fixed varied from 1.4 to 7 mg. of nitrogen in 100 gm. of soil. Eight organisms fixed over 5 mg. of nitrogen; consequently they compare favorably with the azotobacter in their nitrogen-fixing abilities. To date, however, it has been impossible to grow the organism and get appreciable nitrogen gains in nitrogen-free synthetic media.

Summary

The bacterial activities of soils are greatly modified by soluble salts. The numbers of bacteria ammonifying and nitrifying powers of the "alkali soils" tested are not fully restored by mere leaching of the soil. The extent to which restoration takes place varies with the concentration and kind of salt present. With the soils tested it was found that leaching is less effective in the presence of chlorides than it is in the presence of sulphates. The leached alkali soils studied rapidly fix nitrogen when seeded into Ashby media, when incubated with a soluble carbohydrate, or when kept under optimum moisture and temperature conditions in the greenhouse. In promoting nitrogen-fixation, the sulphates were found to be the most effective of the salts tested. Fifty-six per cent of the organisms obtained in pure culture from Ashby agar fixed nitrogen in soils containing mannitol, some of them fixing quantities which compare favorably with those fixed by azotobacter. The beneficial bacteria of the soil survive for long periods in soils containing comparatively high concentrations of chlorides and sulphates.

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CURRENT LITERATURE

BOOK REVIEWS

Weeds in agriculture

The subject of weeds in agriculture has received by no means the scientific and fundamental study which it deserves, and which has been devoted to many other phases of agricultural science. The problem of weeds is of extreme importance, although they are often accepted as inevitable. The weed books of the world, with the exception of a few excellent monographs, are in the main ancient compilations, inadequate, and not the kind of literature to impress farmers favorably with the efficacy of the scientific weed expert.

The publication of KORSMO's monumental work,[1] therefore, is an event of importance. It is translated by WOLLENWEBER into German from the original Norwegian. This translation into German is a step in the right direction, although an edition in English would give the volume a still broader availability.

The work incorporates the results of 35 years' fundamental research, and easily surpasses in valuable detail, original thought, and practical suggestions anything so far published, to the reviewer's knowledge, in any other language. KORSMO's effort has produced an opportune standard work on weeds which is certain to earn for itself first place in the scientific literature of agriculture in any country.

The material is divided into eight main sections including divisions of weeds into biological groups; the occurrence of weeds under agricultural practices; the injurious influence of weeds by crowding out of crop plants, or through overshadowing; etc. A section is devoted to a discussion of propagation and dispersal, followed by a section dealing with living weed seeds and perpetuation through underground portions of weeds in arable land. Another section deals in detail with the different kinds of weeds: "About 8–10 per cent of the entire vegetation in countries of the northern temperate zone consists of weeds," it is stated. A most important section is devoted to preventive and control measures. Chemical warfare against weeds is very thoroughly dealt with.

The following section summarizes the results of a number of Norwegian experiments on the control of weeds during crop growth, actually carrying into

[1] KORSMO, EMIL, Unkräuter im Ackerbau der Neuzeit, biologische und praktische Untersuchungen. (Weeds in agriculture of modern times, biological and practical investigations.) Translated into German from the Norwegian manuscript by H. W. WOLLENWEBER. pp. 580. *figs. 470.* Berlin: Julius Springer. 1930.

practice the recommendation of section 6 B, III and IV, which afford striking proof of the exceedingly beneficial effects of proper weed control measures.

The final section reviews the whole subject. One series of experiments indicates the practical results from rational weed control. From 854 experiments with cereals, an average increase in yield through weed control of 490Ko. grain and 365Ko. straw per approximately two acres was gained. Similarly in 70 root crop experiments the gain was 9313Ko. of roots and 1916Ko. of tops, while in 102 experiments with potatoes on the average an increase of 4237Ko. of tubers was obtained per hectare (1 acre=0.476 hectare). Cost of control measures, etc., having been carefully calculated and taken into consideration, the author states: "From all this may be deducted with great clearness the fact that the practice of weed control measures pays the farmer."

An instructive world bibliography on weeds is followed by an exhaustive index.

KORSMO has rendered an inestimable service to world agriculture by the publication of his research data. The world owes KORSMO in return a war against weeds, aided by international legislation to prevent further distribution in these days of rapid transit and intimate intercourse, if such measure is not already too late.—H. T. GÜSSOW.

Use of the microscope

A volume[2] has just appeared which is designed to show in detail how to secure the best results with the microscope under all circumstances. It is a study of the combination of adjustments and variations of methods which will add to the perfection of the image in the microscope. The most important of these methods have been brought together from the original literature, and the author has added the results of his own experience.

The introduction lists 62 causes of injury to the microscopical image and the preventives to be employed in avoiding them. There are 26 chapters which treat of such subjects as different types of microscopes, types or kinds of objectives, oculars and condensers, illumination, light filters and screens, the cover glass problem, drawing, photography, care of the microscope, and others. It has a short chapter on fixing and staining microscopical objects, another which lists a hundred microscopical objects of biological interest, and another which gives fifty practical exercises with the microscope.—J. M. BEAL.

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[2] BELLING, J., The use of the microscope. 8vo. pp. xi+315. *figs. 28*. New York: McGraw-Hill Book Co. 1930. $4.